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THE HEAT PRODUCTION OF THE ALBINO RAT. II. INFLUENCE OF ENVIRONMENTAL TEMPERATURE, AGE, AND SEX; COMPARISON WITH THE BASAL METABOLISM OF MAN

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THE respiration chamber and the experimental technique employed in our study of the albino rat, and the method adopted for the presentation of our results have been described in a foregoing paper.[1] In this first section we discussed the effect of fasting upon the heat production of the rat. Practically all of our fasting experiments were made at chamber temperatures of about 26° C. A few were made at a lower temperature. Ideally such tests should be made only at the so-called "critical temperature." Another feature of our research was therefore to determine the influence of differences in environmental temperature upon the rat's metabolism.

RELATION BETWEEN ENVIRONMENTAL TEMPERATURE AND METABOLISM

Rubner was the first to point out that there is a close relation between the heat production of animals and the environmental temperature in that the lower the temperature the greater is the heat production. He considered a temperature of 33° C. essential in general for minimum heat production. What is the optimum temperature which should prevail if one is to secure the minimum basal metabolism of the rat? Practice has varied greatly in different laboratories. In some cases the environmental temperatures have been as low as 13° C., but in more recent work, aside

[1] Benedict, F. G., and G. MacLeod, *This Journal*, 1929, 1, 343.

from experiments made particularly to study the tremendous differences in metabolism at widely different temperatures (as was done by Giaja[2]), there has been a disposition[3] to have the experimental temperature not far from 25° C. Hári[4] and Aszódi of the Budapest school are inclined to use 27° or 28° C. Even in those laboratories specializing in rat studies the environmental temperatures have by no means been uniform and measurements of the basal metabolism have frequently been made at relatively low temperatures. From the standpoint of comparative physiology basal metabolism should be measured only at the critical temperature and only experiments made at or near this critical temperature should be considered.

Comparison of the Metabolism at High and Low Temperatures

With our apparatus it was possible to alter the experimental temperature considerably by the simple expedient of blowing cold air over the glass cover or putting an electric heating unit under the chamber. A number of experiments were made particularly to study the influence of differences in chamber temperature on the metabolism of the same rat, and in order that the only variant would be the chamber temperature, all the experiments were made 16 hours after food. The results of eighteen series of such experiments, in which the metabolism was studied at chamber temperatures ranging from as low as 10.9° C. to as high as 31.7° C., are shown in Figure 1. Here the heat production per square meter of body surface per 24 hours is referred to the experimental temperature, and each curve represents the data for an individual rat.

Figure 1 shows clearly that the heat production decreases as the temperature becomes warmer. A number of experiments made with a small range in temperature, from 25° to 32° C. (not plotted in Figure 1) indicate that at 28° C. and above the metabolism was essentially constant, although it was in general perceptibly higher at 26° than at 28° C., with much the same rate of increase noted in the longer series of experiments recorded in Figure 1. It is evident, therefore, that the so-called "critical temperature" of the rat is at 28° C. and that no experiments to determine the basal metabolism should be made with this animal at an environmental temperature lower than 28° C.

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[2] Giaja, J., *Ann. de Physiol.*, 1925, 1, 596.

[3] Honda, T., *Biochem. Zeitschr.*, 1927, CLXXXV, 173.

[4] Hári, P., *Biochem. Zeitschr.*, 1924, CLII, 448; Aszódi, Z., *Biochem. Zeitschr.*, 1924, CLII, 458 and 472.

According to the curves in Figure 1 there is considerable difference in the level of the metabolism of the individual rats at the different temperatures, but for the most part the rats which have the higher metabolism at 30° C. also have the higher metabolism at 12° C. As will be shown like-

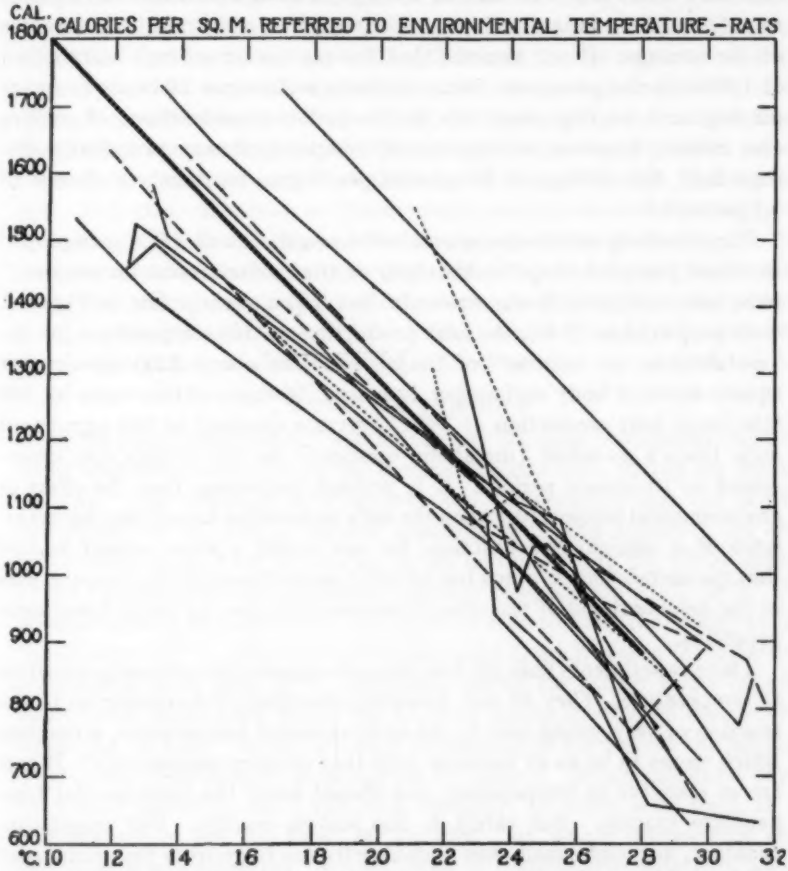

FIG. 1.—Heat production of male and female rats per square meter of body surface per 24 hours, referred to environmental temperature.

Each curve represents the data for an individual rat studied 16 hours after food. The heavy black line from 10° to 28°C. represents the average trend of the metabolism with changing temperature.

wise in subsequent discussion on the influence of sex (see page 385), the majority of the rats at the higher level are males. Although the curves vary in general level, a heavy line indicating on the average the course

of the metabolism referred to temperature has arbitrarily been drawn through them. From this line one would infer that the average metabolism at 28° C. would be not far from 880 calories and at 10° C. about 1800 calories. Thus the difference of 18 degrees in temperature corresponds to a difference in heat production of 920 calories, or 51 calories per degree on the average. If one assumes that the rat has an average metabolism of 1,000 calories per square meter of body surface per 24 hours (a figure playing such an important role in the earlier considerations of surface area where, however, environmental temperature was practically disregarded), this change of 51 calories per degree represents a change of 5.1 per cent.[6]

The extremely cold temperatures in the neighborhood of 0° C., employed in recent years by Giaja[6] in his study of the "métabolisme du sommet," were not considered in our research. But if the average line in Figure 1 were projected to 2° C., the heat production at this temperature (or the "métabolisme du sommet" of Giaja) would be about 2200 calories per square meter of body surface per 24 hours. Division of this value by 880 (the basal heat production at 28° C.) gives a quotient in fair agreement with Giaja's so-called "metabolic quotient" for the albino rat, determined in 10-minute periods. It is perhaps surprising that the effect of environmental temperature upon the rat's metabolism has all the characteristics of a straight-line function, for one would, *a priori*, expect to find that the metabolism is much less affected proportionately by temperatures in the neighborhood of the critical temperature than by much lower temperatures.

These experiments indicate that rats are apparently extremely sensitive to temperature. They do not, however, contribute information as to the reaction of very young rats to the environmental temperature, a reaction which seems to be so at variance with that of more mature rats.[7] If rats are so sensitive to temperature, one should know the experimental temperature exactly. But rarely is this known exactly. Our respiration chamber, although small, was undoubtedly too large from the standpoint of ideal temperature measurements alone. One of the difficulties of determining the average environmental temperature of a respiration chamber

[6] With normal rats Artundo found that the metabolism was approximately 150 per cent higher at 0°C. than at 30°C., and notes that decapsulated rats have less resistance to cold. (Artundo, A., *Compt. Rend.*, 1927, XCVII, 409.) See, also, the fine study on the influence of temperature by Terroine and Trautmann (*Ann. de Physiol.*, 1927, III, especially Table III, p. 436).

[6] Giaja, J., *Ann. de Physiol.*, 1925, I, p. 596.

[7] Gulick A., *Amer. Jour. Physiol.*, 1926, LXXVI, 206.

is to avoid drafts and local heating or cooling of the thermometer. When the bulb of the thermometer is above the animal, one can imagine that the temperature will be unusually high, due to the ascending currents of warm air from its body. On the other hand, the cooling of the glass cover by a blast of cold air would produce a cold air current which would fall and affect the thermometer quickly. Although the thermometer readings do not give the exact average temperature, they are at least of value in indicating differences in temperature. Consequently it is more than likely that the readings of chamber temperature may represent solely relative temperatures and that the records may all be too high or too low. It is clear, however, as Rubner only recently remarked verbally to one of us, that the reaction of the animal, particularly the dog, to the environmental temperature with respect to its metabolism is such as to make the animal practically a thermometer, indicating the temperature by the intensity of its metabolism. What Rubner found true with the dog is in large part confirmed with the albino rat.

Influence of Season

As the study progressed it was noted that the basal metabolism seemed to be somewhat lower during the summer months than during the winter months. The influence of environmental temperature was too slowly recognized by us, and a natural explanation of this tendency for the metabolism to be lower in summer might be that during the winter many of the experiments were made at a chamber temperature of 25° or 26° C., whereas during the summer it was impracticable to keep the temperature much below 28° C. With more attention paid to maintaining the chamber temperature at or near 28° C., especially during the winter, many experiments were made from which the effect of season itself upon the metabolism can be seen. These rats were living most of the time in the laboratory at the temperature prevailing for the season. On experimental days they were placed in the respiration chamber, 16 hours after food, at a temperature of not far from 28° to 29°C., at which temperature the metabolism was measured. A comparison of a series of experiments made at this temperature with a number of rats, both during the winter season (December, January, and February) and the summer season (June, July, and August) is made in Table I. The chamber temperature during the experiment was in all cases at or near 28° C., and the laboratory temperature at which the rats lived when not in the apparatus was, on the average, 21° C. in the winter. The experiments were not all made during the same year, either in the winter or the summer series, but the data were ac-

cumulated over a number of years. For this reason the laboratory temperature in the summer months averaged 27.8° C. with the female rats and 26.2° C. with the males.

With a group of twenty-one adult rats, both males and females, the heat production per square meter of body surface per 24 hours averaged 835 calories during the winter. A group of seventeen male and female rats had an average heat production of 736 calories during the summer. The metabolism was therefore perceptibly lower during the summer season.[8] The temperature of the *chamber*, although varying from 27.8° to 31.6° C., averaged about the same in both the winter and the summer series. During the winter season the number of female rats studied was larger than the number of males, but if the experiments made only on the males during the winter and summer and likewise those made only on the females are compared, one finds that on this basis of comparison the metabolism is again lower in the summer. On the average the decrease in heat production during the summer season may amount to nearly 10 or 12 per cent, even when the rats are all measured at the same environmental temperature and 16 hours after food. This seems to be convincing evidence that season has a distinct effect upon the metabolism of these animals.

The data in Table I also indicate clearly a sex difference in metabolism in that the male rats, both in winter and in summer, have a somewhat higher heat production than the females, although the age element was not held constant in both groups. In general the animals of both sexes were somewhat younger in the summer than in the winter groups, and thus the age factor enters. But even if allowances for age are made (in accordance with the findings on pp. 380 to 385), it is still evident that season has an effect upon metabolism. It may be argued that it is erroneous to average the ages, as given in Table I, since the age range is so great. Further seasonal studies with rats of essentially the same age are justifiable.

In the literature the earliest report dealing with the metabolism of the rat at different times of the year is that of A. V. and A. M. Hill.[9] Experiments were made both in the winter and in the summer, and the temperature of the calorimeter was essentially the same in both series, that is, not far from 14° C. This is a low temperature for the albino rat which, as can be seen from the curves in Figure 1, calls heavily upon its

[8] Although working primarily with mice, P. Pulewka (*Arch. f. Path. u. Pharm.*, 1926, CXII, 82) states that the basal metabolism of mice was found to be higher in summer than in winter.

[9] Hill, A. V., and A. M. Hill, *Jour. Physiol.*, 1913, XLVI, 81.

chemical heat regulation when living in a cold environment. Although the temperature of the laboratory at which Hill's animals lived prior to the actual metabolism measurements is not stated, it is highly probable from reports on certain feeding experiments in their paper that the animals lived most of the time at 14° C. Hence the effect of season, at least so far as environmental temperature is concerned, would not appear.

TABLE I

INFLUENCE OF SEASON UPON THE METABOLISM OF THE RAT, 16 HOURS AFTER FOOD, AT ABOUT 28° C.*

Winter				Summer			
Rat	Age	Body weight	Heat per sq. m. per 24 hrs.	Rat	Age	Body weight	Heat per sq. m. per 24 hrs.
	mos.	gm.	Cal.		mos.	gm.	Cal.
Males				*Males*			
1	22	325	1013	22	9	312	801
2	18	375	766	23	5	255	806
3	11	279	857	3	17	324	665
4	9	281	739	24	13	320	686
5	9	267	811	25	7	280	659
6	7	259	925	26	22	343	736
7	2	101	887	27	10	358	811
8	21	195	903	28	4	254	772
				29	4	230	806
Av.	12	260	863	30	4	257	780
Females							
9	6	153	724	Av.	10	293	752
10	6	143	630	*Females*			
11	2	101	890	31	11	211	761
12	6	149	631	32	12	237	644
13	17	203	823	33	8	223	665
14	21	192	914	34	5	178	644
15	21	199	939	35	8	195	660
16	21	180	839	36	8	193	666
17	23	194	831	17	27	182	953
18	19	244	827				
19	16	226	855	Av.	11	203	713
20	23	169	880	Grand Av.			736
21	19	261	851				
Av.	15	186	818				
Grand Av.			835				

* The data in this table represent in most cases results obtained on one day only; with rats 5, 20, 26, 27, and 34 the values are averages of measurements on two days; with rats 17 (winter) and 21 on three days; and with rats 17 (summer) and 18 on four days.

Although most of our rats showed essentially the same picture, that is, a metabolism low in summer and high in winter, occasionally extreme differences are noted. These differences point toward the possibility of individuality among rats. This is strikingly shown in the case of the female rat 17, which was studied in the winter at the age of 23 months and again four months later in the summer. In the winter at an average chamber temperature of 28.4°C. the heat production on three different days averaged 831 calories. At that time the rat had been living at an average laboratory temperature of 21° C. In June of the same year measurements on four days at an average chamber temperature of 28.1° C. showed that the heat production averaged 953 calories. In the summer the average laboratory temperature at which this rat lived was approximately 25.0° C. This rat's metabolism in June[10] is in striking contrast to the metabolism of all the other female rats studied in the summer, for six other females (although not of the same age) were found to have an average heat production of 673 calories, whereas this particular rat had an average heat production of 953 calories.

It is impossible to explain this extremely high value as due to the differences in age alone. The rectal temperature was normal. Pregnancy at this age could not have occurred. The kymograph records show conclusively that the activity was not above normal. The average laboratory temperature (25° C.) at which this rat lived during the four days in the summer was 3 degrees lower than that at which the other six rats lived. This might account for its higher metabolism, and yet on one of the four days the rat had been living the day before at a temperature of 27° C. and the heat production was 922 calories. In any case the laboratory temperature for 24 hours before was always perceptibly above the laboratory temperature for the winter experiments. Experimentally the protocols seem free from error, for most satisfactory, normal respiratory quotients were secured in every period of measurement. Another possible explanation is that this rat was used for a large number of experiments (nineteen between December and June), which may have made too great a tax upon it. The rat lost 25 grams in weight the first month, but thereafter its weight remained essentially constant. We believe, however,

[10] Although our discussion of the influence of the season is based upon data for groups, the critical reader might be inclined to compare the winter and summer results for this rat in Table I and would find that the picture for this individual rat is strikingly at variance with that for the group. The only other rat studied both in the winter and in the summer was the male rat 3, which had a high metabolism in the winter compared with that in the summer.

that in general these animals recuperate rapidly from 17-hour fasting experiments.

Perhaps the best lesson to be learned from the experience with this rat is the importance of an autopsy at the end of a series of basal metabolism measurements. In the present cooperative work of the Nutrition Laboratory with Yale University on albino rats and particularly with the Department of Genetics at Cold Spring Harbor on pigeons, autopsies are frequently performed. It is to be regretted that the autopsies made in the research here reported did not include rat 17, for the high values found with this animal might have been explained by an autopsy. A further important point brought out by the experiments with this rat is the value of having some sort of standard for the basal metabolism of the albino rat. If a rat shows a metabolism obviously higher or lower than the average rat, it is obvious that this animal is not suitable for respiration experiments, particularly when subtle, superimposed effects are to be investigated. By making preliminary basal metabolism measurements and in case of marked deviation from the standard rejecting the animal, much time and effort may be saved.

Even if this exceptional rat is included in the average for the summer experiments, however, the general picture is clearly that of an increased metabolism in the winter months as compared with the summer months. We are therefore convinced that we have to deal here with a real seasonal effect and are inclined to believe that this effect is explained perhaps wholly by the environmental temperature[11] at which the rats were living prior to the respiration experiment.

To what extent seasonal variation in the metabolism of rats should enter into basal metabolism researches is yet to be established. Only recently a definite seasonal effect with humans has been reported.[12] In experiments in which the influence of long-continued feeding on some special diet or the influence of some other particular factor is to be investigated, one must recognize that a change in metabolism due to change in season may possibly mask or minimize or, on the other hand, intensify the effect of the superimposed factor under study.

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[11] See article by O. Montoro (*Revista de Med. y Cirugia*, Havana, 1927, XXXII, p. 503. Cited by *Jour. Amer. Med. Assoc.*, 1927, LXXXIX, 1185. Original reference not available to us), who states that experimental work done on rats seems to indicate that the basal metabolism is lower in the tropics than in colder climates.

[12] Gustafson, F. L., and F. G. Benedict, *Amer. Jour. Physiol.*, 1928, LXXXVI, 43.

Influence of Previous Environmental Temperature upon the Metabolism of the Rat when Measured at the Critical Temperature of 28° C.

The findings indicating a seasonal effect led to the conviction that, assuming temperature to be the main factor in season, it might be illogical to take a rat out of a relatively cool environment, place it in a respiration chamber at the optimum temperature of 28° C., and consider that the metabolism measured under these conditions is the basal. The next step, therefore, was to provide a known, optimum environmental temperature at which the rat should live for at least 24 hours prior to the experiment. By placing the rat in a 1/4-inch mesh wire cage surrounded by a suitable air bath, the previous environmental temperature could be controlled.

Numerous observations were therefore made of the metabolism of the same rat measured at a chamber temperature of 28° C., after it had been living for twenty-four hours before at a controlled temperature of 28° C., and also after it had been living at the ordinary temperature of the laboratory, which was usually several degrees cooler, averaging 21° C. It is conceivable that the effect of season might not be eliminated by having the rat live for a period of but 24 hours at a controlled optimum environmental temperature and that experiments made in the winter might still show a higher metabolism than those made in summer, even if in both instances the rats had been kept for twenty-four hours before at 28° C. In this series of experiments, however, no experiments were made during the warm weather, and hence season, as such, does not enter into the comparison. Eleven rats were thus studied, one (No. 37) being a male and all the others females. With one of them (No. 17) observations were made on as many as 19 experimental days and with all but one on at least 5 days. The minimum number of days was three, one after the rat had been at 21° C. for 24 hours and two after it had been at 28° C. for 24 hours before. In all experiments the metabolism was measured in the chamber at 28° C. or thereabouts, and the time after food averaged 17 hours, with but slight variations. All the rats were over 11 months old.

The average results for these experiments are given in Table II. The last two columns in this table give a comparison of the heat production measured at a chamber temperature of 28° C., but in the first instance after the rat had been living for 24 hours at 21° C. on the average and in the second instance at 28° C. With but one exception the heat production was lower after the rat had been living at 28° C. In the case of rat 39

there was a slight increase of 13 calories. In a number of other cases, such as with rats 19 and 15, the decrease at the higher temperature is probably insignificant physiologically. On the average there was a perceptible decrease of 37 calories, or 4.5 per cent, at the higher temperature. In other words, the effect of the cooler temperature on the day preceding the experiment was distinctly reflected in the metabolism even though all measurements were made at the same chamber temperature. This implies a stimulated metabolism due to the previous cooler temperature which is sustained for at least 4 to 6 hours (the length of the experiment) after the rat is placed in an environment at 28° C. Przibram reported

TABLE II

HEAT PRODUCTION PER SQUARE METER OF BODY SURFACE PER 24 HOURS OF RATS MEASURED AT 28° C. AFTER LIVING 24 HOURS AT 21° OR 28° C.

Rat	Age	Weight	Heat produced at 28° C. after 24 hrs. at	
			21° C.	28° C.
	mos.	gm.	Cal.	Cal.
37	12	198	871	849
38	12	178	870	828
19	17	235	839	834
39	18	243	767	780
40	19	180	890	840
21	19	263	825	739
18	20	248	789	734
16	22	172	839	765
15	23	190	865	852
20	24	169	882	861
17	25	187	931	885
Av.			852	815

that rats brought from a cold to a warm room have a higher body temperature than rats habituated to a warm room.[13] It is possible that a higher body or cell temperature may explain this after-effect upon the heat production of previous exposure to cold.

Our experiments on this particular subject do not deal with rats younger than 11 months, but the youthful organism should certainly be studied in this respect. Furthermore, our laboratory temperature of 21° C. was perceptibly higher than that which obtains in some rat laboratories, especially in Europe. The problem should be studied further with rats

[13] Cited by Donaldson, H. H., The Rat, Philadelphia, 1924, 2d. ed., p. 152.

maintained constantly at temperatures considerably below 21° C., possibly at the extremely low temperature of 13° or 14° C. used by the Hills,[14] at which it is conceivable that an even more marked reaction in metabolism may be found.[15] The reaction to the previous environmental temperature has, we believe, been too long overlooked.

Our evidence is clear that when rats are taken from a moderately cool environment at about 21° C. and placed in a warm respiration chamber, even at the so-called "critical temperature" of 28° C., the measured metabolism may be perceptibly higher than the metabolism following a 24-hour sojourn at the critical temperature prior to the basal measurements. This is another evidence of the extraordinary sensitivity of the albino rat to environmental temperature. The conclusion is justified that in the future no one should publish the results of basal metabolism experiments on the albino rat, or, indeed, on any animal other than man, without giving both the average environmental temperature during the period when the metabolism was measured and, if possible, the average environmental temperature at which the animal lived for at least 24 hours prior to the test.[16] It is customary to demand that with animals the nutritive state (usually meaning complete withdrawal of food) for 24 hours prior to the test be reported. It now appears that it is likewise important to know the previous environmental temperature. Even as late as 1926 one finds reports of researches on the gaseous metabolism of the rat without any indication as to the temperature of the respiration chamber, much less the laboratory temperature at which the animal was living.

The modern method of testing diets, by feeding the albino rat and noting the growth curve, neglects in most instances the intense reaction of this animal to relatively small differences in temperature. The current custom of having the rat colony live at temperatures as low as 21° C. or even as low as 14° C. is seemingly, in the light of these findings, subject to revision. Although the health and welfare of the animals may not demand that they exist continually at the critical temperature, it appears that the optimum temperature for the growth and the development of rats has not yet been satisfactorily established. It is obvious that in such

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[14] Hill, A. V., and A. M. Hill, *Jour. Physiol.*, 1913, XLVI, 81.

[15] A retarded reaction to rather profound temperature changes is reported by A. Artundo, *Compt. Rend.*, 1927, XCVII, 408.

[16] See Benedict, F. G., and E. G. Ritzman, *Carnegie Inst. Wash. Pub.* No. 377, 1927, 219–221.

considerations the important findings of Przibram[17] with regard to the influence of rather pronounced differences in temperature on growth and activity should be taken into consideration.

The role which environmental temperature may play is recognized in at least two dissertations from Sherman's laboratory, one by Gloy[18] and one by Campbell.[19] In Gloy's dissertation it is clearly shown that the average gain in weight of male rats from the 28th to the 56th day of life was 25 per cent greater in November and December than in May and June. The growth of females for the same interval was 13 per cent greater in November and December. Campbell, in her dissertation, also considers the question of season and shows that the food intake expressed in calories per gram of rat per day was lowest in July and August and highest in December and January. Both these dissertations indicate that the environmental temperature, which is probably the most important seasonal factor, should be recognized in the future. Our own data lead us to emphasize the desirability of this recognition. We believe that the metabolism of the albino rat at the laboratory temperature prevailing in each laboratory should be known. The consumption of food will bear a close relation to this temperature and this animal with its tremendous reaction to changes in temperature, as evidenced by its metabolic activity, must alter correspondingly its food intake if "normal growth curves," which form the basis of innumerable researches at the present day, are to have any true significance. Extreme care is taken in many laboratories to feed rats a certain constant or standard diet, and nearly every laboratory has its own views on this point. It is here suggested that a standard temperature for such normal growth studies is now imperatively needed.

The fact that the albino rat is characterized by extreme sensitivity to environmental temperature, combined with the singular fact that its metabolism increases with advancing age (as will be shown subsequently), and the fact that it can exist now only under conditions of cage confinement lead one to suspect that this animal represents a special case of breeding none the less important for purposes of control in the laboratory, although occupying a small place in the animal kingdom. An approach to the activity of the wild rat is afforded, it is true, by the use of exercise cages, and metabolism measurements on albino rats accustomed

[17] Przibram, H., Temperatur und Temperatoren im Tierreiche, Leipzig and Vienna, 1923.

[18] Gloy, O. H. M., Vitamin B determination and requirement, with special reference to protein intake, Dissertation, Columbia University, New York, 1927.

[19] Campbell, H. L., Growth, reproduction and longevity of experimental animals as research criteria in the chemistry of nutrition, Dissertation, Columbia University, New York, 1928.

to considerable physical activity are much to be desired. There are innumerable animals the metabolism of which has not yet been adequately studied and results upon which would be of great value in comparative physiology, but in lieu of the extensive observations already made on the albino rat it would seem to be imperative that the wild Norway rat should also be studied, if only primarily for comparison with its highly inbred, domesticated mutant, the albino rat.

Influence of Age

In the early studies of various animals the possibility of differences in metabolism caused by age was not considered. At the present day it is clear that with man, whose metabolism has perhaps been the best studied, the effect of age is pronounced. Thus, with new-born infants the metabolism is very low. At the age of about one year the maximum metabolism is attained, either per unit of weight or per unit of surface area. Thereafter it falls off rather rapidly until the age of about 15 years, and from there on until old age there is a steady, though slight decrease in metabolism. Little evidence regarding the age factor has been obtained with the albino rat, and in our research it therefore seemed desirable, particularly in consideration of the rapid rate of growth of the rat, to study the influence of age in considerable detail. Ideally such studies should be made upon the same rat from birth to old age. It was not practicable to do this in our series, and we have resorted to observations upon individual rats at different ages, although not infrequently the same rat was studied at two or more ages.

The experiments best suited for a study of the influence of age would be those made at or about 28° C., since in these experiments the effect of environmental temperature would be stabilized, in so far as possible. But in our study of the age factor we erred at first in not recognizing that the higher environmental temperature (*circa* 28° C.) should be used. Our data are therefore separated into two groups, one in which the measurements were made with the temperature of the respiration chamber between 24.5° C. and 26.5° C., averaging 25.7° C., and the other at chamber temperatures of 28.0° C. and above, averaging 28.9° C. Since the observations are more extensive with female than with male rats, particularly at the older ages, the data for females only are used in studying the effect of age. These are plotted in Figure 2 which shows the heat production per square meter of body surface per 24 hours referred to age. The results obtained at the lower environmental temperatures are indi-

cated by dots and those at the higher temperatures by crosses. All data were secured after the rats had been for about 17 hours without food, and in all instances the rats were non-pregnant and non-lactating.

All of the rats under two months of age were studied in groups of from four to eleven and not singly, in order to have the carbon-dioxide production large enough to permit the collection of one gram of carbon

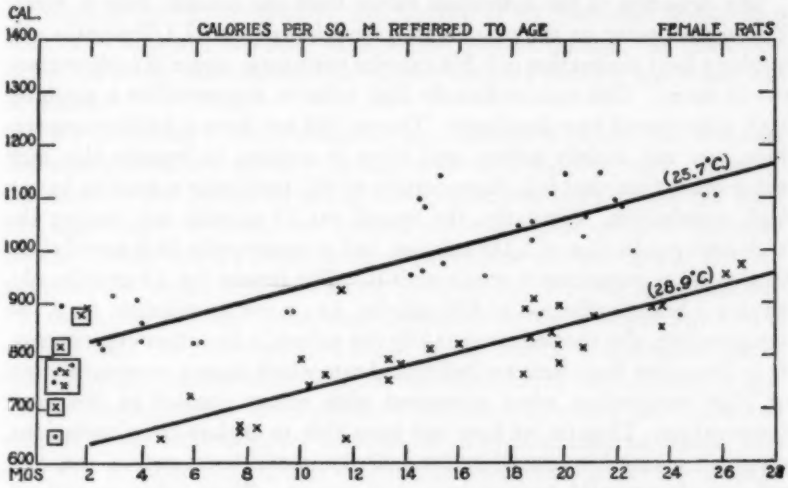

FIG. 2.—Heat production of female rats per square meter of body surface per 24 hours, referred to age.

The data represented by dots were obtained at an average environmental temperature of 25.7°C. and those represented by crosses at an average temperature of 28.9°C. The dots and crosses enclosed in squares represent mixed groups of male and female rats. The two curves deal with rats over 2 months old.

dioxide in the absorbing vessels in a period of reasonable length. At this early age the sex was not noted. These groups are placed upon the chart for comparison purposes, dots and crosses again being used to indicate the experiments at the low and high temperatures, respectively, and these in turn being enclosed in squares to indicate that the data are based on groups.

Prior to the placing of these groups on the chart, an attempt was made to lay through these several points two straight-line curves indicating the general trend of the metabolism, the one passing through the dots representing values obtained at an average temperature of 25.7° C. and the other passing through the crosses representing the values obtained at

28.9° C. This was done independently by two individuals, and the curves represent the averages of their well-agreeing selections. These straight-line curves deal only with the rats two months of age or over, and it is clear that the average trend of metabolism of the younger animals, especially at the higher temperature, would not conform to the straight-line curve.

The deviation of the individual values from the straight lines is great. The highest point on the chart is that for a female rat 27 1/2 months old, having a heat production of 1,364 calories per square meter of body surface per 24 hours. This extraordinarily high value is supported by a similarly high value noted two days later. The rat did not have a febrile temperature, was not unduly active, and there is nothing to explain this high value other than that it is characteristic of this particular animal to have a high metabolism. Similarly, the female rat 15 months old, having the high heat production of 1,148 calories, had a consistently high metabolism in five other experiments made with it. The female rat 19 months old, having a heat production of 880 calories, i.e., a low metabolism for a low temperature, also showed consistently low values in five other experiments. It is thus clear that there are individual rats which have a persistently low or high metabolism when compared with others studied at the same temperature. Thus far we have not been able to explain these variations, although taking into consideration activity, rectal temperature, sex, age, prior environmental temperature, and season. These wide variations, noted with individual female rats are in conformity with observations made on groups of humans, which will always contain at least one individual having a metabolism considerably divergent from the average. Thus, as was pointed out in the report of a study made recently at Wellesley College,[20] in a group of twenty young women one was found to have a high metabolism, not only at the beginning but throughout the entire series of measurements, which extended over a year. A similar instance was noted with one of the men serving as subjects of the experiments on reduced diet, reported by Benedict, Miles, Roth, and Smith.[21] It is obvious therefore that a study of rats having a metabolism widely divergent from the average will present many problems in physiology suitable for further investigation.

The slopes of both of the straight-line curves representing the average

[20] Gustafson, F. L., and F. G. Benedict, *Amer. Jour. Physiol.*, 1928, LXXXVI, 43.

[21] Benedict, F. G., W. R. Miles, P. Roth, and H. M. Smith, *Carnegie Inst. Wash. Pub.* No. 280, 1919, 516.

trend in metabolism at the two temperature levels indicate clearly that with female rats measured at the same temperature there is a distinct tendency for the metabolism to be higher at the older ages, irrespective of whether the measurements were made at the higher or the lower temperature. Indeed, the slopes of the two lines are essentially parallel. In view of the general feeling that in all animal life old age is characterized by debility and even senility, this finding of an increased metabolism with advancing age is wholly unexpected. Studies through the third year of life, however, are imperatively needed before a definite conclusion can be drawn.

With the young rats one month of age, studied in groups, the influence of temperature is not the same as that noted with the older rats. At 26° C. the data for the groups of young rats (males and females) for the most part agree fairly well with the general curve for metabolism at this temperature level. On the other hand, at the critical temperature of 28.9° C. the groups of young rats have a heat production averaging approximately 140 calories above the metabolism suggested by the straight line. It is obvious that the measurements made at the critical temperature are of more physiological significance in the consideration of the influence of age. At this temperature it would appear that groups of young rats under two months of age have a higher metabolism in general than individual rats of from 4 to 12 months of age. Since with young rats under two months old the withdrawal of food for 17 hours causes a more marked lowering in heat production than it does with older rats (see page 366) or even with rats 6 to 7 months of age, it is evident that very young rats have a specifically high metabolism. This finding, based upon measurements made over a wide age range, is in full conformity with the results obtained by Mitchell and Carman[22] in studying rats over a shorter age range. Thus, they note that the basal heat production of rats from 30 to 40 days of age is distinctly higher than the basal metabolism of rats from 90 to 190 days of age.

But the metabolism even of these groups is not so high as that noted on the average with the rats 20 months old or over. A possible explanation of the increased metabolism in old age as noted by us is that the younger rats (considering now female rats two months old or over and not the very young) are perhaps much quieter than the older animals. The activity records here are of value. For example, the activity records were examined for four different female rats which had a low metabolism at the

[22] Mitchell, H. H., and G. G. Carman, *Amer. Jour. Physiol.*, 1926, LXXVI, 385.

age of from 4 to 8 months and of four other female rats which had a high metabolism at the age of from 20 to 24 months, all being measured at 28° C. and 17 hours after food. There is no evidence that the activity was any greater with the older animals than with the younger. Hence this increase in metabolism is not ascribable to differences in activity but to a real age effect.

Although not discussed in this report, our evidence suggests that lactation and pregnancy may increase the metabolism somewhat, and a possible explanation of the higher metabolism of the mature female rats would be that they were lactating or pregnant. But we are certain that our rats were not lactating or pregnant at the time they were used for our metabolism experiments. Furthermore, the albino rat ceases to breed after the age of 16 to 18 months and hence this explanation cannot be called upon to interpret the high metabolism at the older ages. The point may properly be raised, however, that the repeated periods of pregnancy and lactation during the life of the normal female rat may result in a metabolism during old age lower than that of unmated females during old age. Since all of our female rats had been mated and had experienced pregnancy, we have no data on this problem. But certainly experiments should be made, in which the metabolism of mated and unmated females during advancing age is compared.

Figure 2 is primarily designed to note the effect of age, but the fact that two series of experiments made at temperature levels on the average three degrees apart are the bases for these two straight-line curves makes possible further discussion of the influence of environmental temperature upon the metabolism of the rat. These curves show that even with age and sex the same, the metabolic level is definitely changed by a difference in temperature of only three degrees. At each age the difference in heat production is about 65 calories per degree. It will be recalled that in the special study of the temperature factor (see Figure 1 and page 370) the straight-line curve showed on the average a change in metabolism of 51 calories per degree. We believe it is particularly significant that temperatures varying from the critical temperature by only two or three degrees should have this striking effect upon the metabolism. This accentuates still more the need for being extremely careful not only to record the actual temperature at which animals are studied but to be sure that the environmental temperature is a suitable one for the study in hand.

Differences of opinion may exist with regard to the laying on of these

curves in Figure 2, but no change in their location could, in our judgment, alter appreciably the general picture that with the female rat after 2 months of age the heat production per unit of surface area increases with *increasing age* and that it is approximately 65 calories higher for each degree decrease in temperature from 28° to 25° C.

Influence of Sex

Thus far evidence of a sex difference in the metabolism has been seen in two instances. In the discussion of Figure 1, in which the heat production per square meter of body surface per 24 hours is referred to chamber temperature, it was pointed out that the curves for the males in the series are for the most part above those for the females, suggesting that the males have a somewhat higher metabolism. Also in the analysis of the data dealing with the influence of season the male rats, both in the winter and in the summer series, had a somewhat higher metabolism than the females, although lack of uniformity in age obscures the picture somewhat. Since the males were heavier than the females, it might further be argued that in the calculation of the surface area from the body weight, even if the best known constant were employed, there might be discrepancies. On the other hand, if several males each weighing 220 grams (the average weight of the adult female) are compared with females of the same weight—with due regard to the fact that males of this weight are young and that young females must be selected to avoid the effect of age—it is found that the males have a heat production of approximately 800 calories per square meter of body surface and the females nearer 660 calories. This would seem to be convincing proof that male rats have a metabolism somewhat higher than females. In other words, these data suggest that the female organism has the lower metabolism.

Theoretically, the males would be the better sex with which to study the influence of age, inasmuch as the factors of pregnancy and lactation would not play a role. Practically, we used such rats as were available throughout the period of the study, and females were far more numerous, especially above the age of 12 months. The data dealing with the influence of sex are presented in Figure 3, which shows the heat production per square meter of body surface per 24 hours of both sexes, referred to age. Upon this chart were placed, first, the two curves representing the course of the female metabolism with advancing age, these curves being transferred directly from Figure 2. The available measurements for the males were then plotted, using the same basis for selecting the data, namely,

observations made about 17 hours after food and at the two different temperature levels. As in Figure 2, the dots represent experiments made at or about 25° C. and the crosses experiments at or about 29° C. The two average temperatures at which the male rats were studied were, respectively, 25.6° and 29.4° C., or within 0.5° of the average temperatures at which the female rats were studied.

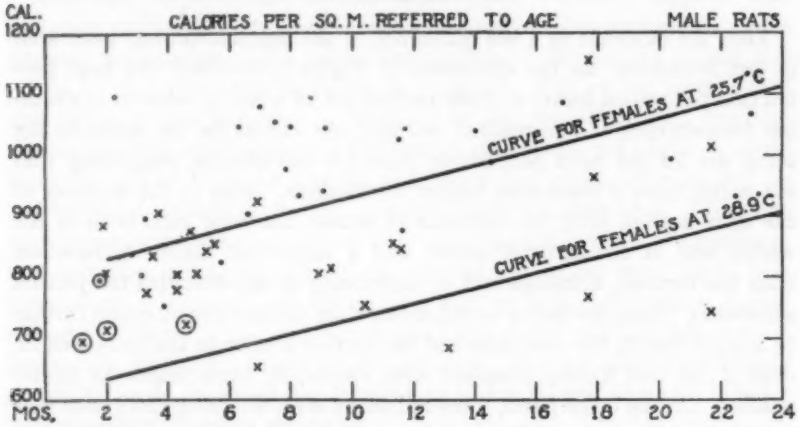

FIG. 3.—Heat production of male rats per square meter of body surface per 24 hours, referred to age.

The data represented by dots were obtained at an average environmental temperature of 25.6°C. and those represented by crosses at an average temperature of 29.4°C. The dots and crosses enclosed in circles represent groups of males. The upper curve indicates the average trend of the metabolism of female rats at 25.7°C. and the lower curve that of female rats at 28.9°C. These two curves are derived from Figure 2.

On this chart the data for four groups (each of three or five animals), all males, have likewise been plotted, the plotted dots or crosses being enclosed in circles to indicate that they represent groups of males. Of these groups three were comprised of the same rats each time. Three of the groups were studied at the higher temperature, and of these two were two months of age or younger. One group of three just a little under two months of age was studied at the lower temperature. These groups as a whole have a much lower metabolism than do the individual males of the same age, particularly individual males at about 4 1/2 months. One could easily argue that this is due to the fact that the animals in the groups huddled[22] closely together and thus presented less surface for heat

[22] See the interesting discussion of nest building as a thermo-regulative activity, presented by E. F. Kinder. *Jour. Expr. Zool.*, 1927, XLVII, 117.

loss. On the other hand, these observations were made at a high temperature, 29.4° C., at which temperature huddling is least effective, theoretically.

The striking difference between the metabolism of the groups of young males in Figure 3 and the metabolism of the mixed groups in Figure 2 is difficult to explain. From the data on males only, one would be justified in assuming that the metabolism of these very young rats, when compared to the older rats, is distinctly low, whether they are measured at the lower or the higher temperature. But the mixed groups in Figure 2 show a relatively high metabolism compared to the older rats, when measured at 29° C. It is obvious that further studies at this age, particularly of individual rats, must be made. In Figure 3 the location of the values for the three groups of males at the higher temperature suggests that any curve drawn through the data for males would certainly not be a straight-line curve below the age of 4 months, no matter what the rest of the age range would indicate. Similarly the one group of three rats at the lower temperature is distinctly below any straight line that could possibly be applied for the observations at the lower temperature.

Before considering the question of sex, the slight evidence regarding the influence of age furnished by the observations on individual males is worth considering, although admittedly the data are deficient. From the plotted data in Figure 3 it can be seen immediately that the number of males over one year old is small. Furthermore, there is a great disparity between the two series of experiments in that at the lower temperature most of the observations occur between the ages of six and twelve months and practically all the observations on rats less than six months of age were obtained only at the warmer temperature. It may be reasoned that to lay on a straight-line curve either through the dots or the crosses on this chart is illogical, because the data are so scattered. If a line were to be drawn through the individual points at the lower temperature, we believe it would in all probability have a slightly upward slope, but by no means as great as that noted in the curve for the females. The same is probably true with regard to any line which might be drawn through the crosses. So far as this uncertain evidence goes, therefore, it does substantiate the finding with female rats, that metabolism increases rather than (as in the case of man) decreases with age, although with males there are few measurements available for discussion beyond the age of 12 months.

Another factor which can be touched upon at this point is that of environmental temperature. The dots are, as a whole, obviously much

above the crosses. If the two straight-line curves for males, mentioned above, were to be laid on this chart, we would find that the curve representing the experiments made at an average temperature of 25.6° C. would be perceptibly above that for the series made at 29.4° C. In other words, a male rat measured at 29.4° C. would produce, on the average, about 150 calories less per square meter of body surface per 24 hours than he would if measured at 25.6° C. Since the difference in temperature here represents 3.8° C., the difference in heat production would be approximately 35 calories per degree. This, it is true, is considerably less than that found with the females of 65 calories per degree. One might infer that males react less to temperature than do females, but this is contrary to the findings in Figure 1 which shows that with extreme temperature differences the straight-line curves for males have practically the same slopes as those for females. In spite of the fragmentary data for males, however, the results can certainly be looked upon as giving confirmatory evidence that each degree fall in temperature between 28° or 29° and 25° C. is accompanied by an increase in heat production of from 35 to 65 calories per square meter of body surface per 24 hours.

The effect of sex is the most important factor to be discussed in connection with Figure 3, and comparison of the plotted dots and crosses for males with the two straight-line curves for females indicates that in practically all cases the males have a distinctly higher metabolism. Only occasionally are the male data below the curves for females. However, little can be said regarding the two sexes above the age of 12 months.

If one were to attempt to estimate quantitatively the difference between the metabolism of male rats and that of female rats, perhaps the best method would be to use some hypothetical curves such as those mentioned above for the male rats. The laying on of such curves (the legality of which we doubt as much as any one) is complicated by the paucity of data after the age of 12 months. If the curves were confined to the data for rats 14 months old and under, we would find that the male curve at the lower temperature would lie approximately 100 calories above the female curve and nearly parallel to it. On the other hand, at the temperature more nearly approximating the critical temperature, that is, comparing the lower curve for females in Figure 3 and the crosses for males, under 14 months, the male curve would be nearly horizontal and would tend to approach the female line, with an average difference, for example, at the age of 8 months of approximately 100 calories. This estimate is extremely uncertain and probably a quantitative measure of

the sex difference is unjustified at this stage of the accumulation of experimental data. That there is, however, a sex difference in the nature of a higher metabolism with the males up to at least 14 months of age, irrespective of whether the metabolism is measured at 25° or 29° C., is, we believe, incontestable.

For this comparison of sexes it would have been better to have obtained all the data at or near the critical temperature of 29° C., to have measured a much larger number of rats in the second year of life, and to have given more heed to the possible effect of season. These omissions are distinct defects in our data, and we are probably not justified in any discussion of male rats beyond the age of 12 or 14 months. Up to this age, however, the higher metabolism of the males, the tendency for the metabolism of males to be higher with advancing age, and a difference in the heat production of males of not far from 35 calories per degree difference in temperature are definitely shown. Thus, so far as the data for male rats go, they completely confirm the more specific findings with the females.

The kymograph records show that the older rats are not more active than the younger. Hence their greater metabolism is not explained by activity. The kymograph records likewise show that the males are not more active than the females, so far as activity during the respiration experiment is concerned. It is a well-known fact[34] that in revolving cages females are much more active than males. This activity may accentuate the sex difference, for increased activity prior to the experiment or as a regular life factor would tend to increase the metabolism of the females. Our rats, however, were not given an opportunity for excessive activity during cage life, and hence no records of cage activity were made.

The Metabolism of the Moribund Rat

In this series of experiments which covered so many years and included so many animals, it happened occasionally that rats were removed from the apparatus at the end of an experimental period in a clearly moribund condition. Rats suspected of being in poor health were never used, but occasionally a rat supposedly normal at the beginning of a series of experiments would subsequently develop signs of disintegration and we believe that in these cases the metabolism measurement during the last period which preceded death is worthy of record. Thus, we have three

[34] Hitchcock, F. A., *Amer. Jour. Physiol.*, 1925, LXXV, 205.

instances of rats which were studied for one or more days, apparently in good condition, and then, for some reason, died shortly after the experiments. Under these conditions a pronounced fall in the metabolism was invariably noted, at times extraordinarily low.

A female rat, studied 24 hours after food on October 28 and again during an 88-hour fast from November 2 to November 5, had up to the sixty-fourth hour of fasting and at an average chamber temperature of 25.5° C. a fairly constant carbon-dioxide production of 3.6 cc. per 200 grams of body weight per minute. After it had fasted 88 hours, at which time it was very lethargic and in a moribund condition, its metabolism was again measured at 23° C. during an experimental period of 2 hours and 29 minutes. During this time the animal produced but 0.7 cc. of carbon dioxide per 200 grams per minute or approximately one-fifth the normal amount. The rat was alive when taken out of the respiration chamber, but was found dead the next day. In another instance a male rat was studied intermittently on four days between December 2 and December 10. When measured 16 hours after food at a chamber temperature of 26° C., the carbon-dioxide production per 200 grams per minute was 3.0 cc. On the last day, after the rat had been 40 hours without food, a 2 1/2 hour period of measurement at a chamber temperature of 21.6° C. showed that it was producing only 0.6 cc. The rat was alive when removed from the chamber at the end of the experiment. The rectal temperature, however, had fallen so low that it could not be read on the scale of an ordinary clinical thermometer. It died a few hours later in the laboratory. Another female rat, 16 hours after food and at a chamber temperature of 26.5° C., produced 3.5 cc. of carbon dioxide per 200 grams per minute but the next day, 40 hours after food, in a period of 2 hours and 39 minutes, at a chamber temperature of 24.0° C. it produced 1.2 cc. or about one-third as much. This rat died the next day.

That the chamber temperature was low in the last experiment in each case, when the rats were in a moribund condition, was in large part due to the fact that the heat production of the animal itself, which plays a large role in keeping the chamber warm, had fallen off to such a degree that the ordinary means employed to control the temperature had failed to hold the temperature up. It is clear that in these instances we had to deal with a moribund rat whose vitality and whose heat production were low. The rectal temperature, when taken, was likewise low. The exact cause of death is not known in all cases. Although it was occasionally coincidental with prolonged fasting for 40 hours or more (which might support

Bijlsma's contention[25] that the rat withstands fasting poorly), our extensive series of experiments on fasting rats has shown that rats can easily withstand a fast of 40 or even 70 hours. The cases cited above are simply fortuitous instances in a long series of experiments, but since the occasions when moribund animals have actually been studied in a respiration chamber are few, the observations are deemed worthy of record as an indication of the tremendous fall in metabolism during the terminal stages of life.

The Basal Metabolism of the Albino Rat

In earlier papers on this subject it has been common for writers to give an approximate value indicating the basal metabolism of the albino rat particularly for comparison with the basal metabolism of other animals. Not many decades ago an average value for man was given, but it is now well known that the value for humans varies with age and sex. From our study thus far, it can be seen that no one figure can be given for the basal metabolism of the albino rat, for with this animal there is evidence of a definite influence of age, of environmental temperature, of season, and without doubt of sex. Some of our data, however, do give a hint as to possible average figures which might be used. According to experiments made at temperatures between 25° and 32° C., for example, there is an indication that the curve for the metabolism flattens out at 28° C. and above, and that the average metabolism at these temperatures would be not far from 775 calories per square meter of body surface per 24 hours. These experiments, however, represent both males and females, and no differentiation has been made for sex. A clearer picture of the basal metabolism, which takes sex into account, is shown in the lower curve in Figure 2, indicating the experiments made with female rats at or above 28° C. An average value for *all* of these females at this temperature would be not far from 800 calories. But as the slope of the curve in Figure 2 shows, the age of the rat plays an important role, and the normal basal metabolism of the female albino rat at 28° C. or above may range from as low as 650 calories with the young animals to about 900 calories with the older animals. This range is too great to permit of using a general average figure without reservations. It is not impossible that temperatures higher than 28° C. may prove to lower the basal metabolism still further. Goto,[26] however, finds that at temperatures over 28° C. there is an in-

[25] Bijlsma, U. G., *Verslagen en Mededeelingen betreffende de Volksgezondheid*, August, 1926, 7.
[26] Goto, K., *Biochem. Zeitschr.*, 1923, CXXXV, 107.

crease in metabolism and considers that the minimum heat production of the rat obtains at 28° C., being on the average 898 calories per square meter of body surface per 24 hours.

We would point out again that in our series of experiments the metabolism was usually measured in periods of two and one-half hours. Undoubtedly somewhat lower values would be found in shorter periods with the absolute minimum of activity, such as the 6– to 20–minute periods with complete muscular repose secured by the techniques of Giaja, Mitchell and Carman, and Bijlsma. It is highly probable that, on the average, our values for female rats could be lowered by a round figure of 10 per cent to bring them nearer the true basal value, uncontaminated by any activity. The metabolism of the young rats mentioned above would thus be lowered to somewhat under 600 calories, and the average for all female rats given above as 800 calories would be lowered to essentially 720 calories.

Although our evidence shows that the male rat has a somewhat higher metabolism than the female rat, it is difficult to apply a percentage correction for difference in sex. But apparently, so far as our results go, the sex difference is somewhat more pronounced below the age of 12 or 14 months than thereafter and probably the best estimate that can be made at present for the difference in metabolism due to sex, suggested in an earlier section, is that the metabolism of male rats under 14 months of age is about 12 per cent higher than that of female rats of the same age. This correction for sex would therefore raise the value suggested above as a standard for female rats (i.e., 720 calories) to about 800 calories for male rats 14 months of age or under.[37]

It is obvious that in the use of any standards for comparison the type of experiment must be taken into consideration, and if one can be assured of short periods of complete repose, the lowest values should be used. Since, as is usually the case, however, in rat experiments fairly long periods are necessary, it is probable that for purposes of comparison the higher standards are more logical. We therefore suggest that the lower curve in Figure 2 (page 381), in lieu of better findings, be tentatively used as indicating the basal metabolism of the female rat when the technique necessitates a long period of measurement. When short periods are

[37] Mitchell and Carman (*Amer. Jour. Physiol.*, 1926, LXXVI, 391), discussing rats between the ages of 90 and 190 days, conclude that the metabolism of males is 5 per cent above that of females.

possible, values 10 per cent lower would be better, and for males between 2 and 14 months of age the highly questionable additive correction of 12 per cent should be applied to the values for females.

The space devoted to the discussion of the basal metabolism of the rat may perhaps not seem justified, and yet such discussion enables comparison of the metabolism of the albino rat with the metabolism of other members of the animal kingdom. In such a comparison knowledge of the true basal metabolism is imperative. In the recent studies of the albino rat reported by Mitchell and Carman[28] a basal metabolism of 600 calories per square meter of body surface per 24 hours was suggested as the normal for males and 571 calories as the normal for females. Insofar as the differences due to sex are concerned, our findings are in general in accord with those of Mitchell and Carman. The fact that in computing the surface area they used a constant of 11.36 as compared with the constant of 9.1 employed by us explains why their values are lower than ours. At first all of our computations of the body surface were based upon the Mitchell and Carman constant, but subsequently, in view of the uniformity in the constants found in the laboratories of Rubner,[29] Giaja,[30] and more recently Lee,[31] we adopted what we believe to be the more correct factor of 9.1. This materially increased our heat values per unit of surface area. In an earlier publication issued by one of us the statement was made that at 30° C. the heat production of the albino rat per square meter of body surface per 24 hours was 580 calories.[32] This was followed by a statement (made before all of our results were accumulated and at a time when we were dominated by the belief that the metabolism would decrease with increasing age) that we estimated that the adult rat would produce nearer 500 calories. These two statements are wrong because based upon the use of the Mitchell and Carman factor for body surface, which is wrong, and because we have found that the metabolism of the rat increases rather than decreases with age.

Comparison of the Basal Metabolism of the Albino Rat With the Basal Metabolism of Other Animals

Supposed intensity of metabolism of small animals. Throughout the literature one finds continually statements that the metabolism of small

[28] Mitchell, H. H., and G. G. Carman, *Amer. Jour. Physiol.*, 1926, LXXVI, 397.

[29] Rubner, M., Die Gesetze des Energieverbrauchs bei der Ernährung, Leipzig, 1902, 281.

[30] Giaja, J., *Ann. de Physiol.*, 1925, I, 607.

[31] See Benedict, F. G., and G. MacLeod, *This Journal*, 1929, I, p. 361.

[32] Benedict, F. G., *Bull. Soc. Sci. d'Hygiène Alimen.*, 1927, XV, 201. See, also, Lee, M. O., and E. F. Van Buskirk, *Amer. Jour. Physiol.*, 1928, LXXXIV, 325.

animals is much more intense than that of large animals. That the canary bird may have a heart rate of 1,000[33] per minute and that the adult albino rat, in complete repose, has an average heart rate of 300[34] per minute are facts which of themselves might suggest a very high metabolism. Another factor is the cell temperature. With birds particularly it was believed that the high cell temperature is productive of a high metabolism. Recent studies on wild birds[35] have not borne out this belief. Thus, a group of large wild birds with a high cell temperature (for the most part over 40° C.) were found to have a basal heat production per square meter of body surface averaging but 874 calories. This fact seems to indicate that a high cell temperature does not of itself increase the metabolism. The rat has, without doubt, a body temperature slightly higher than that of man. But the body temperature of small animals like rats is obtained only with difficulty. To hold a rat on the back with the abdomen exposed to a cool room temperature is of course illogical.[36] The excellent monograph of Przibram[37] leaves little to be desired, however, with regard to information concerning the rat's body temperature and it shows that the cell temperature of the rat is not unduly high.

The only standard for estimation as to whether the metabolism of the albino rat is low or high is obviously the comparison of the rat with other animals and, in the first instance, man. It is assumed that the average adult man has a basal 24-hour heat production of 925 calories per square meter of body surface, and that the adult woman has a heat production of 850 calories.[38] We have found that the female albino rat has a basal metabolism of about 800 calories with cage activity or possibly 720 calories with complete muscular repose. These are values approximately 150 calories lower than the basal values for humans and challenge

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[33] Buchanan, F., Annual Report Smithsonian Inst., 1910, p. 487; *Science Progress*, London, 1910, No. 17, p. 60.

[34] Hoskins, R. G., M. O. Lee, and E. P. Durrant, *Amer. Jour. Physiol.*, 1927, LXXXII, 621.

[35] Benedict, F. G., and E. L. Fox, Bicentenary Number *Proc. Amer. Philos. Soc.*, 1927, LXVI, 511.

[36] Measurement of the rectal temperature was attempted in all of our experiments. Since the technique of such measurements has not yet been perfected, the records were considered of importance only when wide variations from the normal range of body temperature were noted. These rectal temperature measurements are not reported in our results, but the general statement can be made that in all cases we were dealing with rats having a normal range in temperature.

[37] Przibram, H., Temperatur und Temperatoren im Tierreiche, Leipzig and Vienna, 1923, p. 117. His article contains extensive literature citations.

[38] Harris, J. A., and F. G. Benedict, *Carnegie Inst. Wash. Pub.* No. 279, 1919, 178.

attention immediately, in the first place, because of their approximation to the human metabolism and, secondly, because of the fact that the metabolism of the female rat is low rather than high when compared with the metabolism of women.

On the basis that the rat lives at a rate thirty times as fast as do humans,[39] a rat six months of age would correspond to a human approximately 15 years of age. The basal value for children from one year to 15 years of age would average nearly 1,000 calories per square meter of body surface,[40] and for rats, particularly during complete muscular repose, nearer 700 calories. Thus, even in spite of its shorter life cycle, small size, and high heart rate, the rat's metabolism is distinctly low rather than high. This generalization deals with the average adult rat. It is to be regretted that individual rats have not been studied by us in a period corresponding to the first year of life of the child, to secure data for comparison with the striking changes in the child's metabolism during this time. Our data for mixed groups of rats show that the metabolism of the group at 29° C. is higher at the age of one month than it is for individual rats from two to eight months old, but is lower than the metabolism of rats a year and one-half old or over.

With the more mature female rats the average basal heat production (with complete muscular repose) is not far from 720 calories. This, although 15 per cent lower than the average basal heat production of the adult woman (850 calories), certainly approaches the basal value found with women. To some writers this approximate agreement between the metabolism of the albino rat and the metabolism of the adult woman, who has a body weight about three hundred times greater than that of the rat, may seem a confirmation of the so-called "surface area law." A steer, however, with a body weight ten to twelve times that of woman, has a basal metabolism per unit of surface area (1300 calories[41]) 50 per cent greater than that of woman. The mouse, according to Giaja,[42] has an average basal heat production of 1270 calories per square meter of body surface, at temperatures of 32° to 33° C., and the rat of 835 calories. Using the Rubner constant of 11.4 in the Meeh body-surface formula,

[39] Donaldson, H. H., The Rat, Philadelphia, 2d. ed., 1924, p. 32.

[40] Benedict, F. G., and F. B. Talbot, *Carnegie Inst. Wash. Pub.* No. 302, 1921, 174–175.

[41] Benedict, F. G., and E. G. Ritzman, *Carnegie Inst. Wash. Pub.* No. 377, 1927, pp. 203 et seq.

[42] Giaja, J., *Compt. Rend.*, 1925, xciii, 646.

Kraus[43] in his admirable book has computed the careful observations of Aszódi[44] on the mouse, and finds that the heat production at a temperature of about 30° C. averages but 540 calories per square meter of body surface.

Just what factors determine basal metabolism are by no means clear. The Nutrition Laboratory has long maintained that a causal relationship between the surface area and the heat production, based upon the loss of heat from the surface to the environment, does not hold. As a result of the interesting anatomical studies of Dreyer and his associates,[45] in which the surface area of the body, the cross sections of the trachea and the aorta, and the blood volume (a measure of mass) were shown to have a close relationship, it was believed that perhaps the calculated surface area is a better index of the active protoplasmic mass in two different animals than is the body weight. The senior author (F. G. B.) wishes to admit at this point that the pungent criticism of Carman and Mitchell[46] against this use of the body surface calculation is thoroughly just. It is clear that this reasoning, applied to the albino rat, leads to absurd conclusions. Writers setting forth the Nutrition Laboratory's belief in the significance of the protoplasmic mass fail to consider, however, the second factor clearly stated in our consideration of this subject,[47] namely, the stimulus existing to metabolic activity. In other words, metabolism is not a simple function of protoplasmic mass but involves also the stimulus. Because of the extraordinarily high heart rate and the rapid blood circulation of these small animals it is clear that any stimulating factors in the blood would be carried to the cells at a much more rapid rate and possibly with a different concentration than would be the case with humans. In considering metabolism from the standpoint of comparative physiology it is therefore not at all unlikely that we may have to take into consideration not only protoplasmic mass but also another factor thus far usually wholly neglected, namely, the *stimulus* to this mass. We may have an approximate measure of this stimulus in the heart rate and possibly in the blood volume, and in the future undoubtedly factors in blood chemistry will enter.

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[43] Kraus, E., Lehrbuch der Stoffwechselmethodik, I. Teil, Methodik des Energie-und Stoffwechsels, Leipzig, 1928, table 19d.

[44] Aszódi, Z., *Biochem. Zeitschr.*, 1921, CXIII, 79.

[45] Dreyer, G., and W. Ray, *Phil. Trans.*, Series B, 1909–10, CCI, 133; ibid., CCII, 191; G. Dreyer, W. Ray, and E. W. A. Walker, *Proc. Roy. Soc.* Series B, 1912–13, LXXXVI, 39 and 56.

[46] Carman, G. G. and H. H. Mitchell, *Amer. Jour. Physiol.*, 1926, LXXVI, 380.

[47] Benedict, F. G., *Jour. Biol. Chem.*, 1915, XX, 280.

Addendum

Through an oversight citation was not made of the series of experiments carried out by Cori and Cori (State Institute for the Study of Malignant Disease, Buffalo, New York) on rats, in which they studied the fate of sugar in the animal body. Both the Haldane method and the closed-circuit method, employing the principle of Fridericia, were used and values are reported for the oxygen consumption, the carbon-dioxide production, and the respiratory quotient. The experiments were made under especially favorable conditions for securing basal metabolism measurements, i.e., the rats were kept for 24 hours previous to the metabolism experiment as well as during the experiment itself at a temperature between 27° and 28°C. The respiratory quotient (non-protein) of the normal, control rats which had fasted for 48 hours, averaged 0.71. A seasonal difference was observed in the fasting respiratory quotients, the average value from October to May being 0.713 and the average from May to October 0.685. The fasting quotients below 0.70 are attributed to ketonuria, which is ascribed to the seasonal change in the fat metabolism of the rat. (See Cori, C. F., and G. T. Cori, Journ. Biol. Chem., 1926, LXX, p. 557; ibid., 1927, LXXII, p. 615; ibid., 1927, LXXIII, p. 555; ibid., 1928, LXXVI, p. 755.)

Summary

The heat production of the albino rat, measured 17 hours after food, decreases as the temperature becomes warmer and at 28° C. and above is essentially constant, thus indicating the critical temperature at which measurements of the basal metabolism should be made.

Season has a distinct effect upon the rat's metabolism, for the heat production is perceptibly lower (on the average 10 to 12 per cent) in the summer than in the winter. This effect is explained perhaps wholly by the difference in environmental temperature.

Rats studied at 28° C. after living 24 hours at this same temperature have a lower heat production (on the average 4.5 per cent) than rats studied at 28° C. after living 24 hours at 21° C.

Female rats over 2 months old, mated but not studied during pregnancy or lactation, showed an increasing heat production with advancing age, both at 26° and at 29° C. With male rats the data, although not so numerous, especially beyond the age of 12 months, indicate the same trend in metabolism. The increasing metabolism with these rats cannot be ascribed to increased activity, to lactation, or to pregnancy. The repeated periods of pregnancy and lactation during the life of the normal female

rat would, however, be expected to result in a lower metabolism during old age. Experiments to throw light on this problem are needed with both mated and unmated females during advancing age.

Male rats have a distinctly higher metabolism than female rats, up to at least 14 months of age, both at 25° and at 29° C.

A tremendous decrease in metabolism was noted with three rats which were studied up to within a few hours of death.

The strikingly high or low metabolism observed consistently in a few individual cases stresses the importance of careful autopsies and indicates the value of having some sort of standard for the rat's basal metabolism, in order to recognize quickly the possibility of an animal being physiologically abnormal.

The female albino rat, 2 months old and over, at 28° C., has a basal metabolism, with cage activity, averaging about 800 calories per square meter of body surface per 24 hours, and with complete muscular repose averaging approximately 720 calories. The basal heat production of male rats of the same ages may be estimated to average about 800 calories, with complete repose. Thus, in spite of its shorter life cycle, small size, and high heart rate, the rat has a metabolism on the body surface basis distinctly low as compared with that of humans.

In comparative physiology the influence upon metabolism of both the active mass of protoplasmic tissue and the *stimulus* to this mass should be taken into consideration. The heart rate and the blood volume may possibly serve as approximate measures of this stimulus.

THE UTILIZATION OF FATTY OILS GIVEN PARENTERALLY*

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INTRODUCTION

THE question whether fat, introduced into the body by ways other than the digestive tract, can be made available for nutritive purposes, has both a scientific and a practical interest. Physiologically it is important to know to what extent the fat molecules so administered must be altered to become suitable for processes of metabolism and to ascertain what capacity the organism possesses for making the requisite changes. Fat has frequently been introduced parenterally, both in animal experiments and clinically, as a seemingly favorable solvent for drugs, or as a vehicle for substances of diagnostic value in X-ray work. Because of its high potential energy content fat has also been injected at times when it has been found necessary to attempt to nourish patients by other than the customary alimentary means.

A survey of the work that has been done in this field shows that experiments have been conducted with a wide variety of fats. Many different species of animals and some persons have been given fat injections for one reason or another. Sometimes the fat has been introduced intratracheally, intrapleurally, or intravenously; in the other cases intramuscularly, intraperitoneally, or subcutaneously. In attempting to review the literature covering the use of fat given parenterally, it seemed more valuable to summarize the work that has been done in the past from the standpoint of the criteria that were employed by the investigators to judge whether or not the injected material had been utilized.

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* The data presented are taken from the dissertation submitted by Martha Koehne to the Graduate School of Yale University in June, 1928, in partial fulfilment of the requirements for the degree of Doctor of Philosophy. The work was done under tenure of the Mary Pemberton Nourse Memorial Fellowship awarded by the American Association of University Women for the year 1925–26, and a Sterling Fellowship granted by Yale University for 1926–27. Acknowledgment is made of the technical assistance given by Olive Hinegardner and Margaret Coghill, and by others mentioned in the text, and of the many valuable suggestions contributed by Professors A. H. Smith and G. R. Cowgill.

Analyses of Extracted Fats as a means of Studying the Disappearance of Injected Foreign Fat from Tissues

Large quantities of butter were injected subcutaneously into dogs by von Leube (1895). After periods of time varying from seven-and-a-half weeks to three-and-a-half months, the amounts of fat that could be excised from the belly, back, around the heart, and from the mesentery were determined, and the Reichert-Meisel number of the fat from each of these sources compared with that of fresh butter and of the fat normally found in the same areas in untreated dogs. Several of the animals were starved before the injections and received insufficient food afterwards. Von Leube's conclusion was that such injected fat could be used in nutritive emergency but was not normally available in any significant amount for the needs of the animal.

Binet and Fleury (1925) injected olive oil, peanut oil, and castor oil subcutaneously into the posterior paws of dogs. The animals were killed twenty-one to fifty days later and the tissue excised from the paws that had been injected; and, as controls, from those not injected. Fat was extracted from all these sections and determinations were made of the acid number, iodine number, and saponification number of each extract. The values were compared with the corresponding numbers for the oils before injection. The acid numbers of the fat extracted from injected paws were four to five times those of the oils that had been used, with no significant changes in the other values, except what could be explained on the basis of simple mixtures of dog fat and injected fat. Binet (1925) ventured the theory that the change in acid number might be evidence of an active lipase secretion by mononuclear leucocytes. The fat absorption was very slow.

In studies recently made by Carter and Malcolm (1927) with subcutaneous and intraperitoneal injections of mutton bird oil into cats and young rats, the authors determined the iodine number of the fat at the site of the injections, of the colored fatty tissue near the mammary gland, and of normal fat. The animals were killed three to seven days after the last injection. The deep yellow injected fat had been transported to some extent by the lymphatics, but there was little chemical change found in the fatty deposits. Injections resulted in irritation, which set up inflammatory processes leading to the engulfing of the oil by the leucocytes and its subsequent removal as a foreign body.

In 1911 Mills reported a series of observations made on cats that had been injected subcutaneously with various clear oils and with ten per cent emulsions of these oils. The materials used were olive, cottonseed, sesame, peanut, and corn oils, lard, and human fat. He determined the absorbability of the injected oil,—the proportion of cat fat to foreign oil present, compared with the amount injected,—by studying the iodine numbers of the separate alcohol-ether extracts of the dried samples of various portions of the bodies of the animals, including skin, hair, muscles and skeleton. One series of animals was starved, another group was given a low-protein diet, still another a high-protein diet. In the first two groups it was found that the emulsions were utilized from four to six times more efficiently than the clear oils and provided one half to two-thirds of the daily caloric requirement. Lard emulsions showed the highest degree of absorbability, being over eight times that of plain oils. On a high-protein diet clear oils were absorbed as efficiently as were the emulsions. Mills believed that in general the specific absorbability of an injected oil depended upon the closeness of its chemical resemblance to the fat of the host.

Busquet and Vischniac (1921) concluded that the lung is the chief organ for the removal of fat injected into the blood. One lung was excised from the dog used in a given experiment and later four cubic centimeters of olive oil per kilogram body weight were injected into the saphenous vein. Fifteen minutes later the remaining lung was removed. The fat was extracted from each lobe and ten times as much fat found in the second lobe as in the first. By comparing the iodine numbers of olive oil and of the fat extracted from the second lobe, the two were thought to be identical. The authors stated that lung tissue had nine times the fat-fixing power of the liver and thirty-five times that of the kidney.

Chemical Analyses of the Blood before and after Fat Injections

In studying the fate of oil injected intravenously, Busquet and Vischniac (1920) determined the fat content of the blood of dogs before and fifteen minutes after the injection of two to three cubic centimeters of oil per kilogram weight of the animal. The ureters and the bile duct were ligated and still no increase was found in the blood fat after injection and no free fatty acids were recovered from the blood. The investigators concluded that the fat must be removed somewhere. Later (1921) they showed that it was retained chiefly in the lung.

Henderson and Crofutt (1905) made daily subcutaneous injections of large amounts of cottonseed oil in a lean dog. They were never able to demonstrate the presence of the oil in either the blood or lymph, using the Halphen test.

In 1923 Roget and Binet made some interesting observations in connection with the fate of olive oil injected intravenously into dogs. The chest wall was opened up and artificial respiration started. Blood fat and lung fat determinations were made at the beginning of the experiment. They tied off the bronchial tube leading to a portion of the lung, its blood supply being left intact. The blood supply to the rest of the body was somewhat restricted. At varying periods of time after injecting oil intravenously, blood fat and lung fat determinations were repeated, comparisons being made with the fat in the lung tissue having full air supply with that from the lobe from which the air was shut off. Roget and Binet found that lung tissue receiving air destroyed fat brought to it much more quickly than those portions whose air supply was excluded, but that in either case lung tissue promptly removed fat from the blood into which it had been injected.

Nitrogen Balance Studies

In 1897 von Sommer injected sterile olive oil on six successive days into a physically sound twenty-year old idiot. The food intake was controlled and all excretions analyzed, in order that observations might be made on the protein-sparing effect of the calories given parenterally. Only on the sixth day did von Sommer secure positive evidence of protein-sparing action. The duration of the experiment was too short for any reliance to be placed on his results.

In 1898 du Mesnil de Rochemont made a total of 500 subcutaneous injections of olive oil in a series of twenty-eight patients, studying the effect of the calories so introduced on nitrogen metabolism. Favorable balances were obtained in starved persons, in both normal and pathological cases, but the author admitted that the large quantities of fat which he injected disappeared very slowly.

Mills and Murlin (1909-10) made nitrogen balance studies on dogs and rabbits before, during, and after subcutaneous injections of lecithin emulsions of cottonseed oil. It was noted that fat injections exerted a somewhat unfavorable effect on nitrogen catabolism in the dog. They compared the protein-sparing effect produced by giving fasting rabbits twenty-five per cent of their daily caloric requirement in the form of fat injections with the effect produced by giving the animals isodynamic quantities of dextrose by mouth. Results showed that under the conditions of the experiment, the fat was much less effective than the sugar.

Negative results were also obtained by Henderson and Crofutt (1905) who daily for ten days injected 180 cubic centimeters of sterile cottonseed oil subcutaneously into a dog fed a diet of lean beef. The animal was unable to use this storehouse of food to an extent sufficient to prevent steady nitrogen losses. Failure to recover on autopsy approximately two-fifths of the amount of oil injected, representing an average of thirteen cubic centimeters that disappeared daily, was accounted for in large part by losses from the skin which was very greasy.

Zondek and Bernhardt (1925) tried to lessen protein catabolism in twenty uremic and pre-uremic old persons by injecting daily twenty to forty cubic centimeters of olive oil intraperitoneally. They secured some improvement but stated, however, that the good effects did not last long and that injections could not be kept up indefinitely.

Histological Examination of Tissues Impregnated with Parenterally-Administered Fat

Some investigators have made examinations of lung tissue after intratracheal fat injections. One per cent osmic acid has been generally used for staining the sections. By such studies, something has been learned about the mechanism and extent of absorption of oils so administered. In 1919 Boissan and Guieysse-Pellissier reported observations on intratracheal injections of olive oil in rabbits. They were able to show a deep penetration of the oil in the finest branches of the bronchial system. These scientists suggested the possibility of the discovery, some time in the future, of a fat-soluble drug which would have a specific inhibitory effect upon the growth of the tubercle bacillus and which could be added to fat and administered intratracheally, thereby insuring its reaching all the alveolar spaces.

Olive oil, with and without carmine, was injected intratracheally into dogs in 1920 by Guieysse-Pellissier. He showed the engulfing of the dye by the epithelial cells and secured a large increase in the number of polymorphonuclear leucocytes, with many eosinophile leucocytes.

Roget, Binet, and Verne (1923) made successive sections of the lung tissue of dogs at varying periods of time after olive oil had been injected intravenously. They were able to demonstrate the positive rôle of the lung in absorbing this fat from the blood, the hypertrophy of the endothelial cells, and the gradual decrease in size of the accumulated fat globules. Similar studies were reported by Gilbert and Jomier (1924).

Examinations of areas into which olive oil and horse oil,—with and without Sudan III—had been injected subcutaneously, were made by Binet and Verne (1925). They used rabbits, dogs, and guinea pigs and made sections of the tissue at periods varying from eight to seventy-two days after the introduction of the fat. An encystment of the oil droplets was found. The outer shell of the cyst was composed of connective tissue fibers and fibroblasts and contained many leucocytes, both polymorphonuclear and mononuclear, with an increased number of capillaries. The leucocytes engulfed the fat but not the dye. Horse oil was found to disappear more rapidly than olive oil, and there was no encystment of its globules.

Olive oil and petrolatum solutions of chaulmoogra oil or its esters were injected into guinea pigs intratracheally and subcutaneously by Corper and Freed (1922). It was demonstrated that olive oil tended to remain in the alveoli, producing mild pneumonic changes, while chaulmoogra oil produced profound and intense changes in the lungs.

In 1927 Pinkerton reported the autopsy findings in six infants and children who had died as a result of lung disorders. He found that in each case an oil had been given to the child when it had been lying down. The oil had been administered either as food or in the form of local nasal applications. Some of the fat had, in each case, entered the trachea and penetrated the bronchial alveoli. There was considerable variation in the resultant condition of the lung, depending upon the length of time the oil had been in the lung and the nature of the oil. Cod liver oil, liquid petrolatum, milk fat, egg oil, and olive oil had been involved. Some of the sections examined showed merely the presence of oil-laden mononuclear cells in the alveoli; others indicated marked nodular fibrosis; while still others,—as in the case of cod liver oil,—indicated necrosis, disturbed circulation, and malnutrition of the tissues. These observations are of special significance in view of the rather general use of intratracheal injections of iodized oils in pulmonary X-ray examinations (Iglauer 1926).

A unique series of experiments *in vivo* was reported by Clark and Clark (1917), in which they injected various fats into the fin of the tail of tadpoles and watched through a microscope what happened in the living animal. They observed that soon after the injection leucocytes came through the walls of nearby vessels, flattening out on reaching the fat globules and becoming pigmented. In a few hours branches from the lymphatics began to grow out to the leucocytes and fat particles. The tips of the branches remained in contact with the leucocytes several days, but no pigmented leucocytes entered the lymphatics. The droplets of oil in the leucocytes became smaller and smaller and gradually disappeared.

Miscellaneous Criteria of Parenteral Fat Absorption

Iodized sesame oil was injected into persons by Winternitz (1903), who judged the extent of absorption of the oil by the amount of iodide in the urine. He assumed that the oil would be utilized at the same rate as the iodine. From the experiments, Winternitz estimated that only twenty or twenty-five calories per day could have been derived from the injected oil.

Henderson and Crofutt (1905) attempted to ascertain by means of the Halphen test whether cottonseed oil was present in the milk of animals into which this oil had been injected subcutaneously. None was ever detected. Tests for its presence in the urine and feces of the animals were also negative.

According to the observations of Mendel and Daniels (1912–13) when fat containing dye is injected intravenously the dye separates from the oil and is eliminated by way of the bile. This would negative the value of the presence of the dye in the stool as an indication of the utilization of fat so injected unless one could prove that separation took place only when the oil was utilized.

Schwartze (1921) injected peanut oil intraperitoneally into several kinds of animals, including rats, mice, guinea pigs, rabbits, and cats. Sometimes the oil contained dye. He studied the amount of free oil left in the abdomen after varying lengths of time, the intensity of staining of different parts of the abdomen with the dye, and the disappearance of the dye from the oil. His animals suffered no ill effects from the injections, but there was relatively slow absorption of the oil that had been so administered.

In 1925 and 1926 Binet reported observations on the injection of fats containing diphenyl anthracene, which was added for the purpose of rendering the oils fluorescent, thus supposedly enabling him to follow their course through the system. This work seemed to demonstrate the slowness of resorption of the parenterally-administered oil.

After injecting from fifteen to one hundred cubic centimeters of oil at intervals into a series of patients, Stüve (1896) was able to report the complete disappearance of the injected oil as shown by the autopsy of some who died a day or two after an injection had been made.

Finally clinical improvement as a result of injection of fat into persons who could not take nourishment by the more customary means has, at times, been used as a criterion. Schick, Fries, Kohn, and Cohen (1923–4) introduced ten to one hundred cubic centimeters of cottonseed or olive oil at each injection, subcutaneously, into young children suffering from chronic nutritional disturbance. Gains in weight and general clinical improvement that followed were believed to have been brought about by these injections. They stated that the first injection was better utilized than subsequent ones and that the most favorable location for injections was the abdominal wall.

Summary of Available Information

Earlier studies have demonstrated that the lung can remove olive oil which has been administered intravenously and that this same oil is widely distributed in the finest branches of the bronchial alveoli after intratracheal injections. It has also been demonstrated that there is a marked difference in the cellular response of the lung to olive oil given intratracheally as compared with chaulmoogra oil, and that horse oil is more efficiently absorbed when injected into animals subcutaneously than is olive oil which is of vegetable origin. By the studies that have been made much information has been gained on the nature of the reaction of cells to a foreign fat.

The extraction of the fat of excised tissues and its subsequent

analysis has proved a favorite method for attempting to estimate the extent of utilization of fat injected subcutaneously. Particular emphasis should be given to the work of Mills (1911) from the standpoint of the variety of fats used and their injection into cats under the varying nutritive conditions of starvation, low and high protein diets. Von Leube likewise tried the starvation method on his dogs in an effort to force the utilization of the butter injected. The general conclusions reached from all the published data have been that oils so administered are utilized very slowly if at all under ordinary conditions of nutrition. Mills' conclusions vary somewhat from this but since they are based in large part on iodine number determinations of extracted fat there is room for doubt as to their validity. There is too much chance, during the process of extraction, for the occurrence in the fat of chemical changes of such a nature that variations in iodine number would result.

The attempt to study the extent of metabolism of parenterally administered fat by means of changes in nitrogen balance as a result of the extra energy so derived, has led to the general conclusion that its utilization is too slow to have any noticeable effect on protein catabolism. Somewhat favorable results were obtained in starving persons by du Mesnil de Rochemont and, for short periods of time, in old persons by Zondek and Bernhardt. Olive oil and cottenseed oil have been the only fats used and animal experiments have been conducted only on dogs and rabbits.

Chemical analyses of blood before and after fat injection have contributed to our belief that the lung is the chief organ for removing injected fat from the blood. Olive oil has been the only fat used and dogs the only experimental animal.

Conclusions as to the successful metablism of an injected fat when based on the utilization or non-utilization of a substance dissolved in the fat before injection cannot, as yet, be given serious consideration. Dyes, iodine, vitamins, and various drugs (Miller 1920) are examples of fat-soluble materials that have been introduced parenterally in oil solution.

There is some evidence from autopsies that oils administered parenterally have disappeared from the subcutaneous areas of persons in a few days. There is also some evidence that injected oil may be of definite therapeutic value in cases of severe undernutrition.

Items about which Further Information is Desired

Binet and Verne's observation in 1925 on the more effective utilization by animals of an injected animal fat (horse oil), as compared with

a vegetable fat (olive oil) makes it seem desirable to test a variety of fats from each group. Are differences in the cellular response to local injections of fat inherent in the source of the fat, whether animal or vegetable, or in the degree of saturation of its fatty acids, or in the size of the carbon chains in the fatty acids of which it is composed? The work of Pinkerton certainly shows the necessity for a more intensive study of the fate of different kinds of fat in the lung especially in view of the rather widespread use of intratracheal injections of iodized oils in pulmonary X-ray diagnosis.

Mills' work in 1911[1] brings up several interesting questions. Will an animal utilize an injected fat only if this fat closely resembles in chemical make-up its own body fat? Is there any connection between the fineness of the state of division of the oil that is injected and its possible utilization as indicated by Mills's findings in regard to the higher absorbability of emulsions over plain oils? Would a high protein diet have a favorable effect on the utilization of parenterally administered fat in other animals than the cat? Is this effect due to the high specific dynamic power of protein? Would any factor which raised the rate of metabolism of the animal likewise favor the utilization of such fat stores? Will starving an animal always force the utilization of deposits of parenterally-administered fat? What influence does the species or size of animal have on the possibilities of deriving energy from injected fat? Would the size of the lymph vessels be a factor? Would different tissues of an animal have varying powers of metabolizing this fat? Is it safe to say that a fat is utilized if a substance dissolved in it at the time of injection has been utilized?

No effort has been made, in the experiments which are now to be described, to observe the effects of intravenous, intrapleural, or intratracheal injections of fatty oils. A few injections were made intramuscularly but most of them have been intraperitoneal or subcutaneous. Likewise no attempt has been made to answer all the above questions. The experiments were planned to add, if possible, to the available information on a few of these topics. The method of study in each series of experiments will be outlined in the reports which follow.

Materials and methods

Fatty Oils and their Preparation

Two animal fats and two vegetable fats were used, of which butter oil and cocoanut oil represent fatty acids that are more highly saturated,

while cod liver oil and peanut oil are composed chiefly of unsaturated fatty acids.

The *cocoanut oil* and *peanut oil* were high grade commercial products. The *butter oil* was prepared by melting fresh butter, draining off the supernatant fat layer, and filtering it through filter paper on a hot water funnel. This clear fat was then melted, cooled down slowly, with frequent stirring, to 26 degrees C., and filtered quickly through layers of cotton in a warm Buchner funnel. The butter oil used in the experiments that follow was that portion which remained liquid at 26 degrees C. The *cod liver oil* was in part an unflavored oil obtained from the E. L. Patch Company of Boston and in part a commercial Squibb product.

Emulsions, consisting of 75 per cent oil, 23 per cent water, and 2 per cent egg lecithin, were prepared for us by Fairchild Brothers and Foster of New York. With the exception of that made from cod liver oil, these emulsions were unstable at refrigerator temperature and deteriorated too rapidly at room temperature to permit their use.

The oils and the cod liver oil emulsion were sterilized by exposure to ultra violet light. A quartz tube somewhat similar in general principle to that used by Fairhall and Bates (1920) was made by the Hanovia Chemical and Manufacturing Company. Fairhall and Bates claimed that oil passing through a quartz spiral of three to three-and-a half mm. bore at a rate of 1.5 liters per hour and exposed to ultra violet light in passage is sterilized without chemical changes in the time required. Figure 1 (page 441) represents a drawing of the tube used in the experiments. It was attached at each end by wire to the mercury tubes of the lamp at a very slight angle and about ten centimeters from the source of the light. The oil dripped through at the rate of twenty drops per minute and spread out in a thin layer over the flat bottom of the tube before running out. A given portion of the oil could not have been exposed to the light longer than ten minutes. Bacteriological tests made at the New Haven Hospital by Edna W. Lyon showed an absence of organisms in the final products used.

The sterilized oils were kept in covered sterile bottles plugged with cotton in a refrigerator. When it was desired to use some of the oil the bottle was removed, placed in hot water to melt or warm the fat, the neck of the bottle was sterilized in a flame, and the desired amount of oil poured into a sterile weighing bottle from which it could be drawn up into a hypodermic syringe.

Hypodermic needles and syringes were cleaned with benzene, then

with 95 per cent alcohol, and lastly with ether. The needles and syringes were kept in an electric oven set at 100 degrees C until just before using.

On the graphs representing the rate of growth of representive animals in the experiments, the time of making injections is always indicated by very short lines projecting upward from the main curve.

Experimental Animals and their Management

Dogs and albino rats were used as experimental animals. The rats were kept over large mesh wire screens to prevent coprophagy; usually they were kept in separate cages. This was especially important in starvation experiments, to prevent eating of cage mates, and in the vitamin studies. The dogs were kept in separate metabolism cages, 30 inches deep, 30 inches high, and 42 inches wide, with the top and upper third of the sides made of open grating. The cages had false bottoms of ¼ inch mesh galvanized wire to catch the feces.

Food was given *ad libitum* to the rats, except in the starvation periods. Tap water was always available. When yeast or cod liver oil were given separately, they were placed on an inverted crucible lid in the cage and were eaten immediately. Food for the dogs was weighed out individually and given at the same hour once a day, but tap water was always available. Carmine (about 200 mg.) was used as a marker for the feces in the metabolism studies with the dogs.

For purposes of irradiation, two or three rats were placed in a tall glass beaker which was put directly under the lamp at a distance of about two feet,—the open end of the beaker being next to the lamp. Initial exposures lasted two minutes, increasing two minutes each day up to twenty minutes, which period was then maintained. Irradiations were made each day except Sunday.

Technique of Oil Injections

Hypodermic needles number 24, ¾ inch long, and number 26, ½ inch long, were used with a two cc. syringe for making oil injections in rats. For dogs a ten cc. syringe and a needle size 18, 1½ inches long were used. Dogs were secured to an animal board in such a position as to permit injection into the desired area. Rats were held gently by an assistant in such a way as to prevent biting or movement of the legs. The operator removed the hair from the area to be injected, clipping as close to the skin as possible. The clipped area was washed with tincture of iodine and then with 95 per cent alcohol and the skin gently lifted up while the needle was inserted. After the injection was completed the skin

was again pinched up on removing the needle and held in this position while vigorous massage at the site of the injection scattered the oil that had been inserted, to prevent subsequent seepage. The skin was finally washed with 95 per cent alcohol. If then any leakage of the oil was observed the skin was again lifted up at the site of the injection and massage repeated. Leakage was seldom noticed after intraperitoneal injections but had to be watched for carefully after subcutaneous injections.

Experiments and Results

The Utilization of Butter Oil and of Cod Liver Oil given Parenterally, with Observations on the Utilization of their Vitamin A Content.

Series A Experiments

The series of experiments reported here was designed to show to what extent young rats deprived of vitamin A can make use of butter oil and cod liver oil, when injected subcutaneously, intramuscularly, and intraperitoneally, and to what extent the vitamin A content of the oils so administered is available to the animals as a cure for or a prevention of xerophthalmia and other manifestations of deficiency of this vitamin.

Historical Review: Most of the researches of this nature that are reported in the literature have been motivated by a desire to study the effectiveness of vitamin A so administered, with little or no attention paid to the utilization of the fat accompanying it.

Blegvad (1923) injected a ten per cent solution of a very potent cod liver oil extract in pure olive oil subcutaneously into a man who had severe ophthalmia as a result of malnutrition associated with cancer of the liver. After two injections the xerosis had disappeared and the night blindness was ameliorated. After six injections the man could see to walk in the evening and could read in the dim light. The author regarded this treatment as advantageous in cases of poor food assimilation associated with ophthalmia. Bloch (1925) used intramuscular injections of vitamin A-bearing fat on a nine year old boy with xerophthalmia. Because of diarrhoea the patient could not assimilate food well enough to permit taking the fat by mouth. Bloch was able to cure the ophthalmia by giving the vitamin parenterally.

An attempt was made by Wollman and Vagliano (1923) to study the utilization of vitamin A in cod liver oil and butter oil injected into rats subcutaneously and intraperitoneally. So many of their animals died soon after the injections that the results are of little value. The small

number that did survive the illness following the injections were later able to grow. Therefore the authors claimed that the vitamin A injected had been utilized.

Nakahara (1926) compared in rats the rate of multiplication of mononuclear and endothelial cells at the site of fat injections, when olive oil alone was employed, with that obtained on injecting olive oil plus "biosterin," a very potent vitamin A concentrate. He reported that the presence of vitamin A definitely speeded up assimilation of foreign fats, through its effect on increasing the number of such fat-engulfing cells. Burrows and Jorstad (1927) related some lipomas to vitamin A deficiency, as a result of observations on the effect of the presence and absence respectively of vitamin A on tumor formation caused by fat injections.

Details of the Writers' Experiments: Series A. Sterilized butter oil and cod liver oil were used. Young rats twenty-one days old were fed the following rations which were devoid of vitamin A.

Modified McCollum Rolled Oats Ration

	Per cent by Weight
Rolled Oats	40
Dextrin	36
Hydrogenated fat*	10
Gelatin	10
Salt Mixture (Osborne and Mendel)	4

The above ration was further modified for rats 15 and 16,[1] by giving them 5 per cent hydrogenated fat* and 5 per cent butter oil, and for rats 12,[1] 13, and 14 by giving 7 per cent hydrogenated fat* and 3 per cent cod liver oil, during the time indicated on the chart.

Purified Casein Ration

	Per cent by Weight
Casein	18
Cornstarch	51
Hydrogenated fat*	27
Salt Mixture (Osborne and Mendel)	4

100 mg. dried yeast given daily to each rat.

The casein was extracted for two 4-hour periods with ether and three 4-hour periods with 95 per cent alcohol. It was then dried, pulverized, and heated in an oven for 36 hours at 110 degrees C, in very shallow layers, with constant circulation of air.

[1] Rats 16 and 12 are represented on Chart I.

* "Crisco"

CHART I

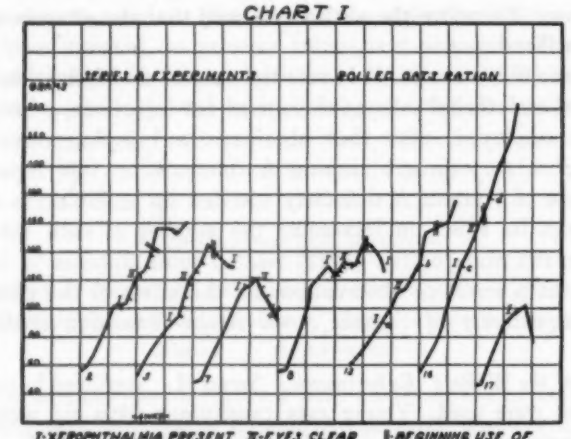
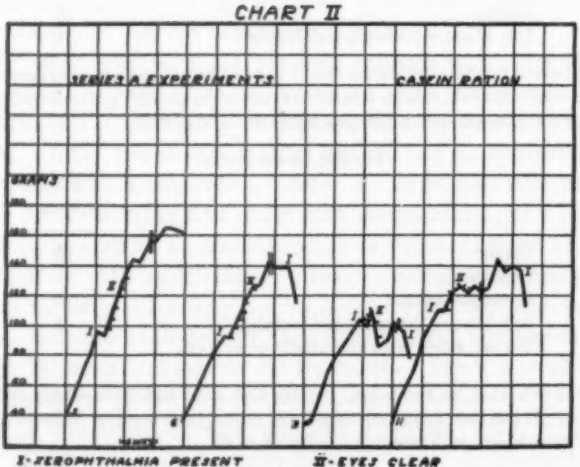

SERIES A EXPERIMENTS
ROLLED OATS RATION
GRAMS
I-XEROPHTHALMIA PRESENT II-EYES CLEAR
|-BEGINNING USE OF IRRADIATED RATION
o—o COD LIVER OIL FEEDING •—• BUTTER OIL FEEDING

CHART II



Charts I and II contain graphs representing the rate of growth, fat injection or fat feeding periods, and the onset and disappearance of xerophthalmia for *representative* animals used. The following summary gives the remaining available data concerning *all* the animals in this series of experiments.

Butter Oil Injections

Rats 1, 2, and 3:*** Subcutaneous injections.

The ophthalmia present at the time the injections were started disappeared during the course of the injections and did not recur before the animals were killed. Rat 1 died of injury received during the last injection. Rat 2 showed a characteristic small abscess at the base of the tongue. The general condition of the rats was good and the lungs were clear at autopsy. In each animal small areas of brownish pasty material were found at the sites of injection, but these were rather small compared with the amount of fat injected. There was no indication of infection in these areas.

Rats 4, 5, and 6:*** Intraperitoneal injections.

In all three cases the ophthalmia present disappeared during the period of injections, but it recurred before the animals were killed or died. On autopsy all three showed infected lungs and abscesses at the base of the tongue. There was no free fat found in the abdominal cavity of rats 4 and 6, but there was a considerable amount of deep yellow encapsulated fat in this area. No fatty deposits or free oil were visible in the abdominal cavity of rat 5.

Cod Liver Oil Injections

Rats 8 and 11:*** Subcutaneous injections.

The conditions of both animals resembled that of rats 4, 5, and 6, as regards recurrence of ophthalmia and the infection in lungs and on tongue. They were in very poor condition when killed. They were dirty and seemed sick. There was little evidence on autopsy that any of the fat had been utilized. It was present in yellowish brown pasty masses at the sites of injection.

Rats 7, 9,** and 10:* Intraperitoneal injections.

Rats 7 and 10 showed recurrence of ophthalmia after its disappearance during the injections. All three animals had pus sacs at the base of the tongue and infected lungs. Both animals showed a great deal of deep yellow encapsulated fat around all the abdominal organs.

Control Groups

*Rats 15 and 16:** Butter Oil Feeding.

In both cases the butter oil feeding period continued only during the time the other groups of animals were receiving fat injections. The xerophthalmia disappeared promptly and did not recur before they were killed. The animals were in excellent condition on autopsy.

Rats 12, 13, and 14:* Cod Liver Oil Feeding.

Rat 12 had inflamed eyes at the beginning of the oil feeding period but the condition cleared up in a few days after the change in ration. Rats 13 and 14 never developed inflammation of the eyes. Rats 12 and 14 had a few spots on the lungs. Otherwise all the animals were in good condition on autopsy.

Rats 17, 18, 19, 20, and 21:* No Source of Vitamin A.

The five animals died showing all the characteristics of deprivation of Vitamin A.

* Rats 2, 5, 7, 8, 12, 16, and 17 are represented on Chart I.

** Rats 3, 6, 9, and 11 are represented on Chart II.

The young rats were limited to one or the other of the above rations for periods of time varying from four to five weeks. From the beginning each rat was exposed to ultra violet light daily to avert rickets. Toward the latter part of the experiment, at the periods indicated on the charts, the basal ration was irradiated instead. This was accomplished by exposing the lot made up, in a very thin layer, for one half hour at a distance of two feet.

The following criteria were used in judging the effectiveness of the treatment given: (1) *The possible observed improvement or prevention* of those symptoms ordinarily associated with vitamin A deficiency such as xerophthalmia and abscesses at the base of the tongue. (2) *Findings of gross autopsy,* appearance of sites of subcutaneous injections and the distribution of oil in the abdominal cavity, as free oil or as fat-like deposits. (3) *Possible distribution* of the oil via the lymph.

Conclusions: Unquestionably the rats were able to utilize the vitamin A carried by the fat. We had better success than did Wollman and Vagliano, for only one of our animals died as a result of an injection, and this loss was due to mechanical injury. With the limited number of animals in each group, those receiving subcutaneous injections of butter oil fared better than those injected intraperitoneally. It seems unlikely that this was due to the ingestion of some of the fat as a result of licking at the sites of injection, although such a possibility is not entirely excluded. There was little evidence that any of the cod liver oil was used. In the butter oil injection series, rat 5 must have used most of the oil received, and rats 1 and 2 probably used part at least.

The Utilization of Cod Liver Oil Emulsion Given Parenterally, with Observations on the Utilization of Their Vitamin D Content.
Series B Experiments

Cod liver oil and a lecithin emulsion thereof were introduced parenterally into a group of young rats that had been on a rickets-producing diet three to four weeks. The purpose of the experiments was to show the use made of the oil and the emulsion under these conditions, and also to what extent the animals were protected against or cured of rickets by such treatment. Histological examinations of bones and tissues as well as gross autopsy findings were used to evaluate the outcome.

Historical Review: Cod liver oil concentrate which was dissolved in either olive oil or glycerol was injected subcutaneously into rachitic

rats by Zucker and Matzner (1923–4). No healing of rickets resulted if the animals were prevented from licking the oil oozing from the sites of injection.

Soames (1924) fed a rickets-producing diet to young rats. The animals were injected intraperitoneally, twice a week for two weeks, with small amounts of cod liver oil which had been sterilized one hour at 120 degrees C. Precautions were taken to prevent seepage of the oil from the point of injection and subsequent possible licking of this oil by the rats. From the beginning one group was given no cod liver oil, another group received injections of cod liver oil, and a third group was given an equivalent dose orally. The experiment was continued twenty-one to twenty-nine days. Bones from each group were then analyzed for water and calcium oxide. Results showed that there was a partial utilization of vitamin D by the group receiving the injections. However, there were conglomerations of small oil globules surrounded by fibrous tissue in the abdominal cavity, showing that the bulk of the *oil* had not been absorbed. Levy-Solal, Christou, and Dalsace (1926) injected rats on a rickets-producing diet with peanut oil and olive oil rendered anti-rachitic by irradiation, and claimed to have had healing effects.

Kramer, Kramer, Shelling, and Shear (1927) demonstrated that the effectiveness of vitamin D taken parenterally depended upon the medium in which it was injected. When vitamin D concentrate was dissolved in ether and injected in rachitic rats, it was effective, but not when in solution in tri-palmitin. Further confirmation of this was given by the work of Wilkins and Kramer (1927), who secured the beginning of calcification and improved blood calcium and phosphorus in two children by giving intramuscular injections of an ether solution of cod liver oil concentrate. Finally, Hume, Lucas, and Smith (1927) rubbed irradiated cholesterol dissolved in ether into small shaved areas of the skin of rachitic rabbits. The animals were then dressed in chamois leather jackets to prevent licking of these areas. The rabbits were kept in separate cages. Radiographs were taken and careful autopsies made. The authors were convinced that the vitamin D so given had been utilized and that the intermediation of the gastro-intestinal tract was unnecessary.

Details of the Writers' Experiments: *Series B*: Sterile, clear unflavored cod liver oil (E. L. Patch Company) and a lecithin emulsion thereof were used as sources of vitamin D. Thirty young rats, at twenty-one days of age, were put on the Steenbock rickets-producing ration consisting of,—

	Per cent by Weight
Yellow corn	76
Wheat gluten flour	20
Calcium carbonate	3
Sodium chloride	1

Vitamins A and B were supplied by the whole yellow corn, but there was no protection against rickets given to any of the rats for from twenty-three to twenty-eight days. The rats were never exposed to direct sunshine. At the end of this preliminary treatment, they were divided into ten groups,—four untreated (controls) and six receiving injections (see Protocols). Injections of both the oil and the emulsion were made intraperitoneally, subcutaneously, and intramuscularly.

The oil injections were given at four different times, three to four days intervening between injections. One-half cubic centimeter of oil or three-fourths cubic centimeter of emulsion were used each time. Intramuscular and subcutaneous injections were made first in the left hip, then into the right hip, left shoulder and right shoulder. Intraperitoneal injections were all made in one small area. All the animals receiving injections were killed eleven to thirteen days after the last injection.

The criteria used in judging the effectiveness of the method of administering vitamin D and the extent and manner of utilization of the oil consisted of: (1) *Gross autopsy findings* as regards the well known signs of rickets, and *histological examinations* (made by Dr. Helen Scoville, Department of Pathology, New Haven Hospital) of the femurs of all rats in the series,—as means of evaluating the degree of rickets present. (2) *Gross examination on autopsy* at the sites of injection of the oil or emulsion to estimate roughly the extent of utilization of the oil, and *histological examinations* made by Dr. Scoville, of body tissues from all rats receiving injections for information on how this foreign material was handled by the body cells. Corresponding tissues from rats killed at the time the injections were started, were examined as controls. The sections examined represented the sites of injection of the oil on hips and shoulders or the fatty deposits around internal organs. The tissue and bone sections were made by Adah I. Baird, Department of Pathology, New Haven Hospital.

Chart III shows the rate of growth and the periods of injection or feeding of Vitamin D-bearing fat for *representative* animals used. The

following is a record of the gross autopsy findings and the results of histological examination of the femur and various body tissues of *all* the animals in this series of experiments.

Control Animals—No Injections

Rats 30, 31, 32, and 34.* Basal diet *ad libitum.*

These animals were killed when the injections in the other rats were started. This group was used as a basis of comparison for all other groups. The degree of rickets was placed at 0, a slight beading of the ribs being the only positive evidence.

*Rats 35 and 36:** Basal diet *ad libitum.*

The two rats lived 45 and 49 days after being placed on the experimental diet and showed before death and on autopsy all the classical symptoms of the most severe rickets. There was complete lack of ossification and loss of structure in the epiphysis of the femur. Cellular outlines were blurred with only a few spicules of bone, very irregular in shape. Degree of rickets ++++.

Rats 55 and 56.* Basal diet for about three weeks, then cod liver oil by mouth during the time the others were receiving injections. One-fourth cubic centimeter was first administered with a medicine dropper every three to four days. Later two per cent was substituted in the ration. Still later ten per cent was substituted for part of the corn in the diet.

There was no gross evidence of rickets. The general condition of the animals was good. There was almost complete ossification of the head of the femur, with open cancellous tissue made up of narrow branching spicules between which were islands of typical bone marrow. Rat 56 showed a few more irregularities than the other. Degree of rickets 0 and +.

Rats 57, 58, and 59.* Basal diet for about three weeks, then cod liver oil emulsion by mouth during the time the other groups of rats were receiving injections. One half cubic centimeter was first fed with a medicine dropper. After a few days two and a half per cent was incorporated in the ration. Still later twelve and a half per cent was substituted for some of the corn in the diet.

There was very little indication of rickets on gross autopsy, but the outward appearance of the rats was very poor. The fur was shaggy, dirty, and oily. On examination of the epiphysis of the femur, there was found almost complete ossification, with only a narrow cartilaginous band running directly across. Degree of rickets 0.

Animals Receiving Injections of Cod Liver Oil

Rats 37, 38, and 39:** Intramuscular injections of cod liver oil.

Gross autopsy findings. Internal organs were in good condition. At the sites of the injections, there were yellow brown pasty masses under the skin or penetrating deep into the muscles. No pus was present but the fore legs were somewhat swollen.

Examination of Femur. There were a few detached islands of cartilage with separate islands of cartilage in the cancellous bone. There were some irregular wide bands of cartilage showing a tendency to merge normally with newly forming cancellous bone. Degree of rickets 0 to −.

Examination of Tissue at Sites of Injection. The immediate reaction was one of tissue necrosis with the appearance of polymorphonuclear leucocytes. The final picture was one of large irregular multinucleated cells and newly forming capillaries. There were some isolated endothelial leucocytes present, probably representing the anlage of the giant cells. These rats were much like rats 40, 41, and 42 except the reactions were more localized. Some of the cells contained oil droplets.

* Rats 31, 36, 55, 57, 38 and 39 are represented on Chart III.

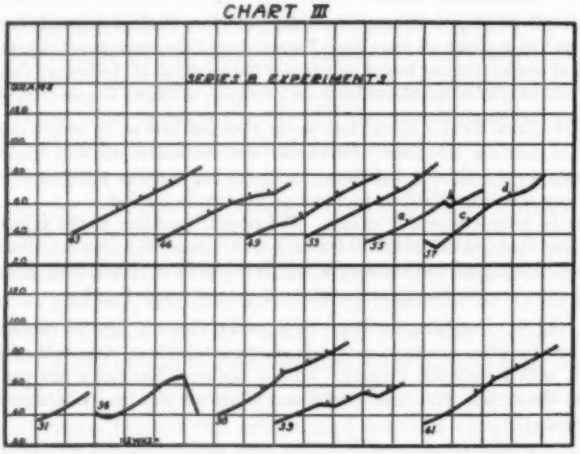
Rats 40, 41, and 42.* Subcutaneous injections of cod liver oil.

Findings were similar to those reported for rats 37, 38, and 39.

Rats 43 and 44.* Intraperitoneal injections of cod liver oil.

Gross autopsy. A slight beading of the ribs was the only evidence of rickets. There was no free oil in the abdomen, but there were large masses of deep yellow encapsulated fat surrounding the abdominal organs, especially around the spleen, on the abdominal side of the diaphragm, and around several lobes of the liver. There were many nodules of this same deeply colored fat-like material along the lymphatics, even as far as the axillae and neck. The rats were in good general condition when killed.

CHART III



Examination of Femur. There were no detached islands of cartilage in rat 44. Degree of rickets 0. Rat 43 showed a few isolated islands of cartilage. The band was quite irregular and showed some projections of the cartilage into the bone. Degree of rickets +.

Examination of Organ Tissue. There was a moderate amount of necrosis with the formation of many cysts. Endothelial cells were quite conspicuous, many of which contained two nuclei. There were large numbers of fully formed giant cells. The greatest cellular reaction was found around the liver, spleen and pancreas.

Animals Receiving Injections of Cod Liver Oil Emulsion

Rats 45, 46, and 47.* Intramuscular injections of cod liver oil emulsion.

Gross autopsy. The spine of each animal was badly deformed and there was moderate beading of the ribs. There was much impregnation of the muscles with yellowish brown pasty masses at the sites of the injections.

Examination of the Femur. There was some calcification of the shell of the head of the bone. The band at the junction of the head and shaft was narrow and much broken up and doubled

* Rats 41, 43, and 46 are represented on Chart III.

on itself. There were projections into the cancellous bone which contained many nests of isolated cartilage cells. Degree of rickets ++.

Examination of Tissue at Sites of Injection. There were large cystic areas formed by collections of oil globules. The walls of the cysts contained masses of giant cells and endothelial leucocytes.

Rats 48, 49, and 50.* Subcutaneous injections of cod liver oil emulsion.

Gross autopsy. Slight deformity of the spine was observed in these rats, with some beading of the ribs and moderate enlargement of the epiphysis at the knee. There were pasty yellowish masses at the sites of all injections.

Examination of Femur. These findings were similar to those in rats 45 to 47. Degree of rickets −−.

Examination of Tissue at Sites of Injections. Rats 45 to 50 showed more necrosis than did those rats that received comparable injections of pure cod liver oil,—rats 37 to 42. In rats 45 to 50, the predominating cells were polymorphonuclear leucocytes with a conspicuous number of giant cells and new capillaries.

Rats 51, 52, 53, and 54.* Intraperitoneal injections of cod liver oil emulsion.

Gross autopsy. The general condition was good in all except rat 54. Autopsy findings, with this one exception, were much as in rats 43 and 44. Rat 54 showed signs of rather severe rickets, but by accident it had received only one injection instead of four.

Examination of Femur. The histological picture was much like that in rats 40 to 42 except for rat 54, which more nearly resembled that found in control rats 35 and 36. Degree of rickets −, in rat 54 it was −−−.

Examination of Organ Tissue. The largest deposits of fat-like substance were seen around the liver, spleen, and pancreas. Cystic areas contained remnants of brightly refracting oil droplets. These areas were surrounded by a large number of newly forming capillaries and irregular giant cells, with a moderate number of polymorphonuclear leucocytes.

Conclusions: Without doubt the rats received considerable protection against rickets from the oil injections. In general the clear oil was more potent than the emulsion. The subcutaneous and intramuscular injections were in all cases less effective than the intraperitoneal injections. Inasmuch as leakage of oil at the site of the injection was almost never seen when the oil was administered intraperitoneally and in view of the precautions always taken to guard against this possibility it seems quite unlikely that any of the oil could have entered the digestive tract by way of the mouth.

Generally speaking the response of the tissues of rats to cod liver oil was essentially that associated with any foreign body. The immediate result was a varying amount of necrosis with an associated polymorphonuclear reaction. The oil remained in the tissue as an inert substance and was gradually surrounded by foreign body giant cells. There is no evidence that any of the cod liver oil had been used as a source of energy.

* Rats 49 and 53 are represented on Chart III.

The Effect of Starvation Versus Good State of Nutrition on the Utilization of Injected Fats,—with Observations on the Influence and Fate of Sudan III Dissolved in the Injected Fats. Series C, D, and E Experiments

It is conceivable that an animal could make use of injected fat only under the enforced physiological conditions of starvation. On the other hand it may be argued that, under adverse conditions of extreme undernutrition, the conversion of parenterally administered fat into energy may be even more difficult to accomplish than would be true normally. Accordingly it seemed worth while to study the effect of the state of nutrition on the utilization of the four fatty oils under investigation. The studies of Schick, Fries, Kohn, and Cohen (1923–4) relating to this subject have already been reviewed.

General Plan: The work reported in this section falls into three general divisions, series C, D, and E, the details of each series being given separately.

In all three series, the percentage loss of body weight for an individual animal was computed from its weight at the time the amount of food given daily was first restricted. With this statement of the loss of weight is also given the number of days that elapsed from the time the food was restricted until death. In some instances enough food was permitted each day to delay death appreciably, in an effort to give the animal full opportunity to draw energy from the store of injected fat.

The method of making the fat extractions is a modification of the procedure that has been used by Anderson and Mendel (1928) in their work on fat formation in animals. Acknowledgment is made to Mr. Wm. E. Anderson for assistance in the conduct of these determinations. The animals were skinned and the hide cut into very small pieces. The abdominal wall was opened up and the free oil extracted from the exposed abdominal cavity with boiling water. The hot water extract was cooled, the fat removed with ether, and the ether evaporated in a tared flask. This is known as *extract a*.

The method of making *extract b* is quoted from Anderson and Mendel (1928),—"The stomach and the intestines were removed in order to exclude any food fat; all of the remaining tissue was comminuted in a meat grinder. The hash was then transferred to a one liter Erlenmeyer flask containing 200 to 400 cc. of distilled water; the flask was covered with an inverted beaker, and placed in an autoclave. In the latter the animal fat was liberated by being rendered in an atmosphere of steam at

a pressure of 38 to 40 pounds for a period of 14 to 15 hours. The autoclave was immersed in a bath of heavy cylinder oil, electrically heated with attached space heaters."

At the completion of the rendering process, the watery extract was filtered while still hot, through cheesecloth, the residue re-extracted with boiling water and filtered. The combined extracts were cooled, extracted with ether and the latter evaporated in a tared flask.

Extract c. In some cases, the residue from *b* was finally extracted with ether, and the extract evaporated, and weighed.

Each of the three extracts was weighed separately and they are designated as *extracts a, b,* and *c* respectively in the descriptions of the experiments to follow. *a* = free oil; *b* = body fat or encapsulated oil; *c* = undetermined residual fat.

In series C and D experiments, the animals had been on the stock ration and were continued on it until the injections were completed. This ration consisted of,—

	Per cent by Weight
Yellow corn or whole wheat meal	51
Yeast	5
Cod liver oil	3
Dried whole milk	30
*Meat residue	10
Sodium chloride	1

During partial starvation periods, the animals usually received

Stock ration	3 grams
Crude casein	½ gram
Cod liver oil	2 drops
Yeast	200 mgm.

If the rats lost weight too rapidly, more stock food and more casein were given. Water was always available.

The special rations used in series E will be described later.

Experiments of Series C

Observations of the extent to which adult rats, while in an excellent state of nutrition, utilize either of the four fats being studied, when introduced intraperitoneally, were made and the results are here reported together with a comparison in the case of cod liver oil, of the extent of

* See Table V, page 434, note **.

utilization when the animals are well nourished and when they are subsequently starved. Observations were also made of the gross autopsy findings as regards the fate of the injected oils, and determinations were made of the amount of fat recoverable in each group of rats, by the method of extraction described.

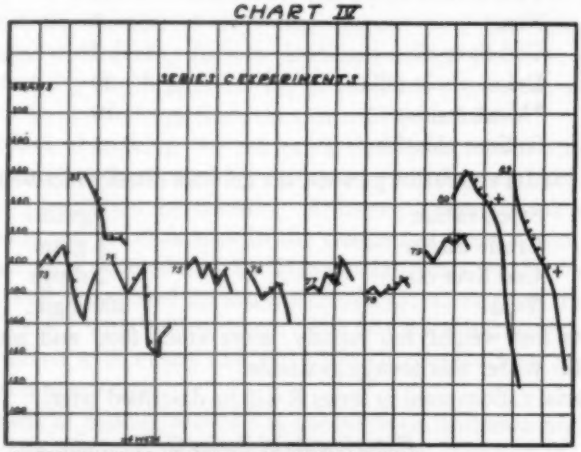
Chart IV gives the weight record, time of injections, and time of beginning gradual starvation for *representative* rats used in this series of experiments. Table I gives the data for the fat extracted from the bodies of all of the rats that were not starved. Table II shows the weight losses of all the animals that were starved. The following is a statement covering the details of treatment, general progress, and gross autopsy findings for *all* the animals used.

Animals Kept in Good State of Nutrition.

Rats 70, 71, 72, 73, and 74:** Intraperitoneal injections of cod liver oil. Food given *ad libitum*.

Rats 70 to 72 received six one cc. injections while rats 73 and 74 received only four in all. Rats 73 and 74 were killed three weeks after the last injection. They were in such poor condition when the last injection was made, that a chance was given for them to recover which they did as indicated on the graph. All the animals lost weight steadily during the injection period. They seemed sick, were very dirty, and there was a reddish brown material around the fore legs of each animal.

CHART IV



All the animals showed masses of deep yellow encapsulated fat in the subperitoneal tissue, these being especially heavy around the spleen, several lobes of the liver, pancreas, and lymph nodes. The lacteals in the mesentery contained considerable yellow material. There was no free oil present in the abdominal cavity. In all cases except rat 72 yellow fat-like masses seemed to fol-

* Rats 73 and 74 represented on Chart IV.

low certain lymph channels and could be found in several subcutaneous areas on the chest, in the neck region, axillae, and groin.

Rat 72 was very edematous when killed. The watery fluid in the abdomen contained small yellow fat chunks. There was marked deposition of this fatty material around the abdominal organs, but none could be found following the lymph channels, as in the other rats.

Rats 75 and 76:** Intraperitoneal injections of peanut oil.

Each rat received four one cc. injections and both were killed one week after the last injection.

*Rat 77:** Intraperitoneal injections of cocoanut oil.

This rat was given four one cc. injections and was killed one week after receiving the last injection.

Rats 78 and 79.** Intraperitoneal injections of butter oil.

Each rat was given four one cc. injections and was killed one week after the last injection.

In case of rats 75 to 79 food was always given *ad libitum*. All animals showed much free oil in the abdominal cavity at autopsy. Rats 78 and 79 showed some yellow encapsulated fatty deposits around certain organs in the abdomen, but this was not true for those receiving injections of peanut and cocoanut oil (rats 75, 76, and 77). There was an abundance of natural body fat in all animals when autopsied.

Animals Starved after Completion of Fat Injections

Rats 80 to 89:** Intraperitoneal injections of cod liver oil.

Each rat received six one cc. injections with food *ad libitum* until the injections were completed. At this point the rats were submitted to the gradual starvation regime described.

Even though these animals died of starvation, there was apparently no decrease in the deep yellow fat deposits in the abdominal cavity. The amount in the lacteals of the mesentery seemed even greater than in rats 70 to 74. The animals were presumably attempting to use this fat for food but were unable to do so at a rate sufficient to keep them alive. There was no true body fat apparent in these animals. Table II gives the percentage loss of weight of each animal during the starvation period. Rat 80 lost 53 per cent of its weight in the twenty-nine days preceding death, yet died with an abundance of yellow fat available in the abdomen. No fat extractions were made in this group of animals.

Discussion and Conclusions. When adult rats daily receiving plentiful amounts of a satisfactory ration were killed shortly after the completion of a series of intraperitoneal injections of different oils and an effort was made to recover the free oil from the body cavity as well as the encapsulated oil and body fat present, varying results were obtained.

Rats 70 to 74 can scarcely be said to have been in a good state of nutrition. Although recovering from the toxic effects of the injections of cod liver oil, they were still very thin,—very little fat of any kind could be recovered from their bodies when they were killed. Judging by the amount of fat recovered by extraction as shown in Table I, the cod liver oil must have been in part disposed of in some way. From the visible amount left in rats 80 to 89 after death by starvation as well as in rats 70 to 74, it is doubtful if such stores had been called upon to provide energy even in nutritive emergency. The method of extraction may have been at fault.

* Rats 75, 76, 77, 78, 79, 80, 85, and 89 represented on Chart IV.

There still remained a plentiful amount of deep yellow encapsulated fat on autopsy. Moser and Wernli (1928) have demonstrated that fat injected or rubbed on the skin may in part be excreted in the urine. No observations of this character were attempted in our experiments. Perhaps this excretory channel may have been responsible for the differences between the amount of cod liver oil injected and the amount recovered. Some may have also been lost through the skin which was always very dirty and somewhat greasy during the cod liver oil injection periods.

Little information can be gained from the data in Table I concerning the utilization of butter oil by rats 78 and 79, since part of this oil was found on autopsy to be free oil,—which would appear as extract *a*,—and part as encapsulated fat, in which case it would be recovered in extracts *b* and *c*, mixed with body fat.

As regards rats 75, 76, and 77, (Table I) that received peanut oil and cocoanut oil injections, in neither case was all the oil that had been injected, recovered in extract *a*. Such fats are never found encapsulated when injected intraperitoneally, as far as can be observed on autopsy. It is possible that some of these oils may have been utilized by the animals.

Encapsulated deposits of cod liver oil are apparently not any more readily utilized by the animals in starvation than when they are not subsequently starved, (cf. Table II and the protocols—rats 70 to 74 as compared with rats 80 to 89). As shown in the experiments of Series B, this form of encapsulation is merely the body's method of handling this type of foreign material. It is probable that an irritating action on the part of oils like cod liver oil hastens the engulfing process. That cod liver oil is locally irritating and toxic is indicated by the marked losses in weight and changes in general appearance that always accompany its parenteral administration.

Experiments of Series D

The negative effect of starvation on the utilization of intraperitoneal injections of cod liver oil made it seem advisable to find out if the same thing would be true for intraperitoneal injections of the other three fats under investigation, and if similar results would be obtained on starving animals into which the oil had been injected subcutaneously. Sudan III was dissolved in all the fats used in this series of experiments in order to make a simultaneous study of the fate of this fat-soluble substance when introduced parenterally.

Details of the Experiment. Each of the four oils injected contained

about one per cent Sudan III. Six injections of one cubic centimeter each were given to a series of adult rats every three or four days. Four groups of animals received intraperitoneal injections and two were given the oil subcutaneously. Partial starvation was begun shortly after the last injection and continued until the death of the animals. At death each rat was autopsied, and extractions of fatty material were made

TABLE I*

FAT RECOVERED FROM BODIES OF RATS RECEIVING FOOD AD LIBITUM

Rats	Oil Used	Amount of Oil	Time since last injection	Extracts a	Extracts b	Extracts c
		cc. per rat	weeks	gm.	gm.	gm.
73–74	Cod Liver Oil	4	3	0.8	3.1	1.3
75–76	Peanut Oil	4	1	4.2	25.1	2.0
77	Cocoanut Oil	4	1	2.6	24.1	1.9
78–79	Butter Oil	4	1	3.5	49.5	4.7

TABLE II*

WEIGHT LOSSES OF RATS STARVED AFTER COMPLETION OF COD LIVER OIL INJECTIONS

Rats	Amount of Oil Injected	Weight Loss	Duration of Starvation Period
	cc.	per cent	days
80	6	26.3	13
81	6	22.0	7
82	6	53.0	29
83	6	30.2	11
84	6	26.3	15
86	6	34.9	17
87	6	34.0	16
88	6	22.2	10
89	6	26.3	13

* See Chart IV and accompanying protocols.

according to the method previously outlined. Careful observations were made of the condition of the animals throughout the experiment together with efforts to determine whether the dye was being excreted in the feces.

During the first part of all the injection periods feces were saved, dried, and extracted with ether to see if any of the dye had been excreted by

way of the bile and intestinal tract, as reported by Mendel and Daniels (1912–13) when fat containing Sudan III was injected intravenously. None was ever recovered.

Charts V and VI give the weight records, time of making injections, and time of beginning the gradual starvation for *representative* animals used. Table III is a record of the treatment and the weight losses from starvation for *each* animal used in the experiments and the amount of fat extracted from the bodies. The following is a statement of treatment and autopsy findings for *all* the animals used.

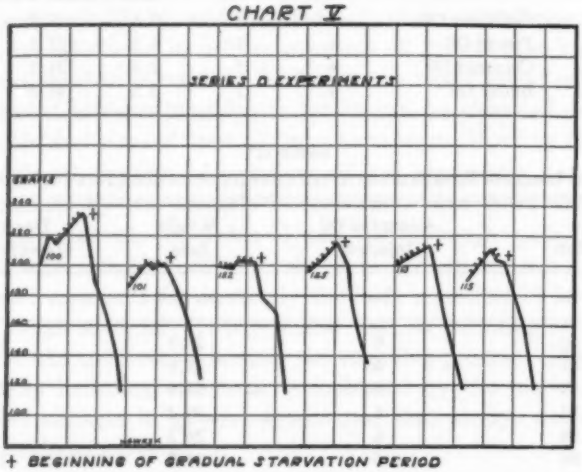
Intraperitoneal Injections

**Rats 100 to 105:* Peanut Oil.

Considerable clear red oil was present in the abdominal cavity, but no true body fat could be seen. There were a few lymph nodes faint pink in color in the abdomen.

**Rats 112 to 115:* Butter Oil.

CHART V



+ BEGINNING OF GRADUAL STARVATION PERIOD

There was a large amount of clear red oil in the abdomen of each rat at autopsy, and also some encapsulated orange yellow fat. Orange yellow lymph nodes were also found in all the animals not only within the abdominal cavity but also in the chest and neck regions and in the axillae.

***Rats 106 to 111:* Cocoanut Oil.

There was no free red oil in the abdomen in rat 107, just a trace in 106 and 108, with larger amounts in the remaining three, that succumbed to starvation much sooner than the first three mentioned. No true body fat was visible in any of the rats at autopsy. There were a few pinkish lymph nodes found.

***Rats 116 to 119:* Cod Liver Oil.

These rats resembled in every respect rats 80 to 89, Series C. One rat not included in the data

* Rats 100,101, 113, and 115, represented on Chart V.

** Rats 106, 110, 118, 119 represented on Chart VI.

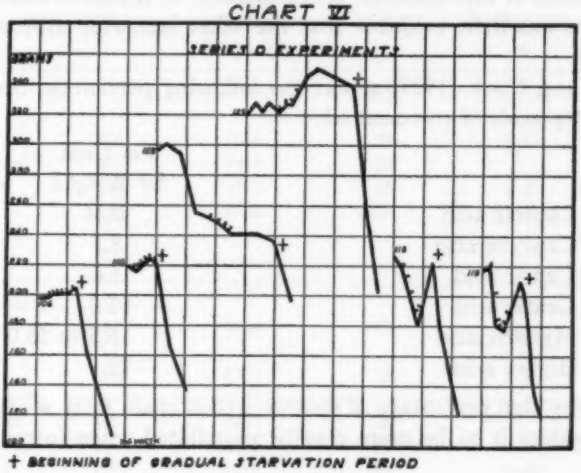
given in Table III died the day after an injection had been made. There was considerable watery red fluid in the abdominal cavity but the fat seemed to be entirely encapsulated and deep orange yellow in color. None of the fatty deposits had the faintest tinge of red color.

Subcutaneous Injections

**Rats 121 to 126:* Peanut Oil.

A rather large amount of clear red oil was present at each site of injection. No body fat was visible and there were no pink lymph nodes found on autopsy.

CHART VI



***Rats 127 to 129:* Cocoanut Oil.

Only traces of red oil could be found on autopsy of these animals, all the body fat was gone, and no colored lymph nodes could be seen.

All of the rats showed a peculiar reddish coloration of the ends of the hair around the head and neck, from the time the injections were started. This continued until they were killed but was very much exaggerated in the case of rats 116 to 119 receiving cod liver oil and Sudan III. The hair of these rats looked as if it was covered with dried blood. Reddish brown material was especially abundant on the hairs around the openings of the genitalia and the anus. After the injections were completed this intense coloration gradually faded out except for traces around the head and neck. This period of deep coloration coincided with the marked losses in weight and seeming illness of the animals during the course of all cod liver oil injections.

Discussion and Conclusions. The two groups of rats which were able to use all of the oil introduced parenterally as a source of energy when impelled to do so by starvation, were those receiving subcutaneous and intraperitoneal injections of cocoanut oil. The more gradual the starvation, the more complete was the disappearance of the oil. Almost no

* Rats 122,125, represented on Chart V.

** Rats 128 and 129 represented on Chart VI.

trace was found of the cocoanut oil that had been injected subcutaneously. That starvation did promote its utilization is seen by comparison of the complete data for rats 106 to 111 and rats 127 to 129 in this series with the records of rat 77 in Series C, in which case there was very slight evidence of its utilization. Part of the intraperitoneally injected peanut oil was probably used,—further evidence to this effect being given in the next series of experiments. (Compare also with rats 75 and 76, Series C). There was little evidence that the other fats were utilized even in starvation.

Taylor and Clark (1927) report the following percentage composition for the fatty acids of cocoanut oil:

	Per Cent by Weight
Caproic acid	0.46
Caprylic acid	8.7
Capric acid	5.6
Lauric acid	45.0
Myristic acid	16.5 to 18.0
Higher acids	22.

Possibly the high percentage of shorter carbon chain fatty acids in cocoanut oil enables it to be more readily assimilated when introduced parenterally.

The dye stayed in solution in the fat in which it was injected and was eliminated only very slowly, except when dissolved in cod liver oil, in which case it separated from the fat very quickly after injection. The dye seemed to be excreted by way of the sebaceous glands. Lymph nodes were tinged with pink color, except when the dye was injected in cod liver oil or butter oil solution. There was no evidence that any dye was excreted by way of the bile and feces.

Experiments of Series E

In this series of experiments the purpose was to compare in rats the utilization of injected peanut oil with that of body fat deposited as a result of eating an excess of peanut oil or starch,—when the animals were in a good state of nutrition and likewise when they were slowly starved.

Details of experiment,—Two rations devised by Anderson and Mendel (1928) were used. With each ration, the animals received daily supplements of 200 mg. yeast and two drops of cod liver oil. During partial starvation periods, each rat received approximately three grams of the

ration it had been receiving in the previous period plus the usual amounts of yeast and cod liver oil. As soon as a rat was killed it was autopsied

TABLE III

EFFECT OF STARVATION ON THE AMOUNT OF INJECTED FATTY OIL THAT COULD BE RECOVERED

Description of Treatment	Number of Rat	Loss in Weight During Starvation	Duration of Starvation Period	Fat Extractions *a*	Fat Extractions *b*
		per cent	days	grams	grams
	100	50.6	33	0.9	0.1
Intraperitoneal	101	44.5	18		
Injections of	102	49.5	26	2.5	0
Peanut Oil	103	44.8	29	1.4	0
	104				
	105				
Intraperitoneal	112			+++	?
Injections of	113	46.4	25	3.1	0
Butter Oil	114			+++	+
	115	43.3	22	1.3	
Intraperitoneal	106	47.0	28		
Injections of	107	52.4	40	0.2	0.7
Cocoanut Oil	108	46.4	26	0.02	0.06
	109			0.6	0.0
	110	40.0	16	some	some
	111	29.0	12		some
Intraperitoneal	116	44.9	19		
Injections of	117				
Cod Liver Oil	118	46.9	23	0.3	
	119	46.0	23		
Subcutaneous (*)	121	35.0	15		
Injections of	122	47.1	26		
Peanut Oil	123				
	124				
	125	46.5	28		
	126	51.0	40		
Subcutaneous (*)	127				
Injections of	128				
Cocoanut Oil	129	38.8	21	None visible	

(*) Subcutaneous injections were made first in the abdomen, then in the right and left hip, right and left shoulder, and last in the center of the back.

and the body extracted by the method already described, in an effort to recover the free oil in the abdomen and the body fat. All injections were given intraperitoneally.

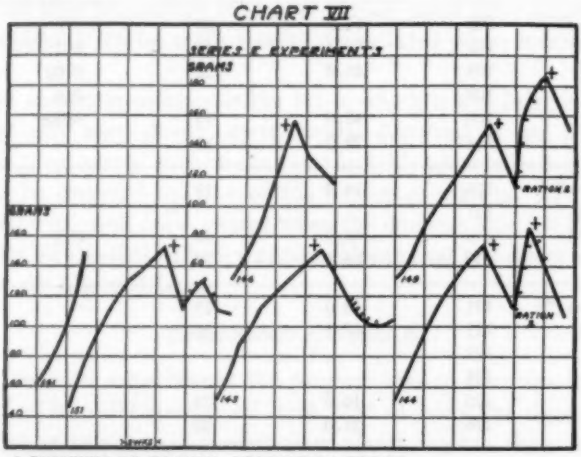
	Ration 1		Ration 2	
	Total Calories	Weight	Total Calories	Weight
	per cent	per cent	per cent	per cent
Dried skim milk	40.	62.8	40.	46.4
Peanut oil	60.	37.2	15.	6.8
Cornstarch			45.	46.8

Ration 1 was fed *ad libitum* to twelve young rats weighing approximately 50 grams until they reached a weight of 150 grams each. Two of them,—*140 and 141*,—were then killed.

The ten remaining rats were gradually starved until they weighed about 110 grams each. When *rats 146 and 147* attained this weight they were killed.

Rats 142 and 143 were held at approximately 110 grams weight by a continuation of the same restricted feeding method, while six one cubic centimeter injections of peanut oil were given at three to four day intervals. They were killed about two weeks after the last injection, still weighing about 110 grams.

CHART XII



+ BEGINNING OF GRADUAL STARVATION PERIOD

After reaching 110 grams in weight, *rats 150 and 151* were given ration 1 in amounts to permit slow gains in weight while they received the six injections of peanut oil. After these were completed they were again gradually starved back to 110 grams weight and killed.

When *rats 144 and 145* reached weights of 110 grams they were put on unrestricted amounts of ration 2 and the series of six injections of peanut oil started. When their weight exceeded 150

grams,—between the fourth and fifth injections,—restricted amounts of ration 2 were given plus the customary supplements, and the animals gradually starved back to 110 grams weight, while the injections were completed. They were then killed.

As soon as *rats 148 and 149* weighed 110 grams as a result of the preliminary treatment, they were likewise given ration 2 *ad libitum* and the injections were begun. This treatment was continued until all the injections had been given, at which point the rats weighed about 185 grams. They were given restricted amounts of ration 2 plus the usual supplements until they weighed approximately 150 grams and then were killed.

Chart VII gives the weight record, injection periods, and time of beginning starvation for a representative rat from each of the above six groups. Table IV gives the amount of fat extracted from the bodies of all twelve of the animals used.

Discussion and Conclusions. On comparison of the amounts of *extract b* secured from rats 140 and 141 with that from rats 146 and 147 it is evident that body fat deposited as a result of liberal consumption of a ration rich in peanut oil disappears during starvation. There was no *extract c* determination made in either case.

TABLE IV

FAT EXTRACTIONS FROM RATS

Number	Weight at Death	Number of Injections	Visible Body Fat	Free Oil in Abdomen	Fat Extractions		
					a	*b*	*c*
					gm.	gm.	gm.
140	150	none	....	none	0.0	7.5	....
141	150	none	....	none	0.0	5.6	....
146	110	none	none	none	0.0*	1.4*	....
147	110	none	none	none	....	....	....
142	106	6	....	....	4.6*	4.6*	4.8*
143	106	6	....	....	....	....	....
150	121	6	....	....	3.8*	0.9*	6.3*
151	111	6	....	....	....	....	....
144	106	6	....	....	4.3*	1.7*	2.1*
145	108	6	....	....	....	....	....
148	149	6	....	....	2.6	1.5	3.1
149	156	6	....	....	1.4	2.3	6.0

* Fat extractions were made on both rats at the same time.

Extracts *b* and *c* obtained from rats in first four groups represented in the above table show the amount of recovered fat that had been stored as a result of eating freely of food rich in peanut

Comparing the amounts of *extracts b and c* that were recovered from rats 144 and 145 with that obtained from rats 148 and 149, it will be seen that most of the body fat that resulted from eating a starch-rich ration *ad libitum* likewise disappears during starvation.

Inasmuch as six cubic centimeters of peanut oil were injected into rats 142 to 145 and 148 to 151, the amount of *extract a* obtained from those animals should be an index of the extent of utilization of this oil, since there was never any apparent encapsulation of this oil when administered intraperitoneally. The most that was ever recovered from any group was 4.6 grams in place of approximately twelve cubic centimeters. The least that was obtained by the method used was 3.8 grams. Apparently some of the oil disappears, but it seems to be utilized at about the same rate when the animals are in a good state of nutrition as when starved, contrary to what was found with cocoanut oil, Series C experiments. (Compare with rats 75 and 76, Table I, series C, and also with rats 100 to 105, Table III, series D.) Peanut oil is utilized much more slowly than cocoanut oil, however. Apparently young rats are able to make some use of peanut oil given intraperitoneally regardless of their state of nutrition.

The Protein-sparing Power of Fat Given Parenterally

In view of the rather inadequate data available in the literature concerning the effect on the nitrogen balance produced by fats administered parenterally, a series of experiments was planned to try to evaluate more fully this method of judging whether or not fats so introduced are sufficiently available sources of extra energy to exert a sparing effect on protein catabolism.

Details of Experiments: Female dogs were fed diets containing either rather liberal amounts of protein or low amounts of this foodstuff. These basal rations were adequate in minerals, vitamins, and bulk. Table V gives the components used. Each new lot of food was analyzed for its

oil. These same extracts in the last two groups of animals represent fat that had been stored from eating liberal amounts of a starch-rich ration.

Rats 142 to 145 and rats 150 and 151 that were killed while weighing approximately 110 grams, had all received intraperitoneal injections of six cubic centimeters of peanut oil. These animals showed rather small amounts of visible hard white fat still present in the abdomen on autopsy, in comparison with rats 140 and 141 and rats 148 and 149. No body fat could be detected in the bodies of rats 146 and 147.

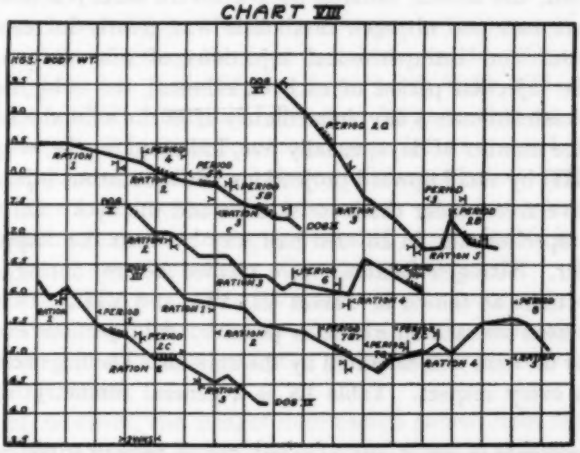
Extract *a* obtained from rats in the last four groups represented in the above table shows the amount of peanut oil that had been injected which was present as free oil in the abdominal cavity at the time the animals were killed.

nitrogen content and the nitrogen values given in Table V represent the averages of these determinations.

Ration 1 was used only for a short period of time as it contained too much fat. No fat injections were made during this period.

Rations 2 and 3,—containing rather low amounts of protein,—were fed at such levels as to provide insufficient calories to insure a sparing effect on food and body protein. Negative nitrogen balances prevailed as shown by referring to Table IX. Experiments conducted during the time animals were on these rations were planned to show whether or not the extra calories provided by the injected fat or the fat added to the ration would decrease this negative balance.

CHART XIII



Rations 4 and 5 contained a higher percentage of protein. These rations were fed at higher levels than were rations 2 and 3 so that the caloric intake per kilogram body weight except for dog 6 was sufficient to insure positive balances (see Table IX). An attempt was made to see if the additional calories provided by parenterally or orally administered fat would increase prevailing positive balances. The dogs gained in weight while on these rations, but lost steadily while on rations 1, 2, and 3. (See Chart VIII).

Definite amounts of food were given to the dogs once a day at the same hour, and excreta were collected quantitatively in order to determine nitrogen balances. The dogs were catheterized at fixed times each day or every other day, just before being fed. There were no cases of infection

of the urinary tract as a result of the frequent catheterizations. Great care was taken to use only sterile instruments. Sterile mineral oil was used on the speculum and on the catheter. The bladder was washed out after each catheterization with sterile water and saturated boric acid solution. Combined fecal excretions for each experimental period,—usually seven days,—were dried, weighed, and pulverized. Determinations of nitrogen were then made on one gram samples.

As soon as an animal's nitrogen excretion became stabilized with the nitrogen intake, some form of treatment was started. Tables VI, VII, and VIII give the record of the various experiments. Data obtained from cod liver oil injections are not recorded because in each case where it was injected, the animal suffered such a severe local reaction that the temperature rose and nitrogen catabolism was greatly increased. Only subcutaneous and intraperitoneal injections of oils were attempted. During the injection period of each experiment, ten cubic centimeters were administered once a day, immediately after the animal was catheterized, for the number of days, usually five, indicated on the weight curves (Chart VIII) by small upward projections. Subcutaneous injections were made in five areas under the loose skin around the neck. Oil was introduced intraperitoneally in an area half way between the diaphragm and the bladder. Nitrogen balances were studied before, during, and after these injections as shown in Tables VI, VII, and VIII. Two different dogs were used for each oil whenever possible. All injections except those of cod liver oil were well tolerated by the animals. The dogs seemed quite normal in every respect. Table IX is a general summary of the data obtained.

In experiments in which oils were fed, ten or twenty cubic centimeters were added to the daily allowance of food, during the number of days indicated in the records. The object was to compare the effect on nitrogen balances of this method of administration with the effect produced by introducing the same oils parenterally.

One dog, not included on Chart VIII or in the tables, was placed for several weeks on a low calorie diet rich in protein. She lost steadily in weight. At the end of this time she was given in the course of one day two 10 cubic centimeter injections of peanut oil containing Sudan III. The following day she was put under amytal anaesthesia and an effort was made to collect lymph from the thoracic duct, to see if the lymph contained any of the dyed fat. There was no trace of pigment in the small sample of lymph secured.

Results and Conclusions: The only experiments that gave consistent evidence of some protein-sparing effect from parenterally injected oil were those in which cocoanut oil was introduced intraperitoneally, (periods 2a and 2b, dog 6; and 2c, dog 4, Table VI). The subcutaneous injection of peanut oil in dog 2, (period 4, Table VII) and its intraperitoneal injection in the same dog in period 5a brought about too small a reduction in the negative nitrogen balance to be of any significance. The most marked sparing effect was produced when the higher protein ration was fed (dog 6, ration 5, Table IX; also period 2B, Chart VIII) at a lower caloric level, than was customary when this ration was used. The dog was in negative balance instead of positive. There was no instance of increasing a positive nitrogen balance as a result of extra energy derived from injected fat. There was likewise no instance of beneficial effect derived from giving these extra calories to the animals in their food, (dog 6, period 3, Table VI; dog 5, period 6, Table VII; and dog 3, period 8, Table VIII). In period 3, ration 3 was used at the customary low caloric level. In period 8, ration 5 was fed at a level to provide enough calories to insure positive balances. It would have been interesting to repeat these experiments giving the ration at a lower level of food intake similar to that used in dog 6, period 2b, to see if this is the factor that accounts for negative results. (See Tables VI and IX).

No final conclusions can be drawn from these results. Further work should be done with higher intakes of protein at lower levels of caloric intake. From the data now available, cocoanut oil seems to be utilized to the greatest extent.

In general, however, the meager information gained from this method of study of the problem is quite out of proportion to the enormous amount of work involved. Fat is too poor a protein sparer, at the best, for small additions to have much effect on nitrogen balances, and it is not practical to inject larger amounts at a time for a successive number of days.

The protocols to this series of experiments give the autopsy findings concerning the fate of the injected oils. Cocoanut oil and butter oil that were administered subcutaneously had completely disappeared by the time the animals were killed (dogs 3 and 4). Dog 1, which is not represented on Chart VIII or Tables VI, VII, VIII, or IX. received five subcutaneous injections of cod liver oil. These injections caused acute irritation with the development of open pustules from which the oil kept exuding for many weeks. Dog 5 which received intraperitoneal injections of cod liver oil did not show the characteristic picture always found in

rats similarly treated and no odor of cod liver oil could be detected in the oil recovered from the abdominal cavity. It is not possible to judge the fate of butter oil and cocoanut oil given intraperitoneally from the autopsy evidence. There was no direct evidence available of utilization of peanut oil from observations made on autopsy of the animals into which it had been injected.

TABLE V

COMPONENTS OF RATIONS USED IN NITROGEN BALANCE EXPERIMENTS
(grams per 100 grams ration)

	Ration 1	Ration 2	Ration 3	Ration 4	Ration 5
Vitamin B Prep.*	5.3	6.6	7.0	7.0	14.0
Sucrose	18.0	21.2	21.0	17.0	10.0
Dextrin	46.7	49.7	56.0	50.0	50.0
Meat residue**	3.3	3.3	6.0	6.0	6.0
Butter	13.3	9.9			
Lard	6.7				
Cod Liver Oil	2.7	3.3	4.0	4.0	4.0
Bone Ash	2.7	4.0	4.0	4.0	4.0
Crude Casein				10.0	10.0
Salt Mixture***	1.3	2.0	2.0	2.0	2.0
Av. Nitrogen	0.58	0.64	1.03	2.44	2.31
Approximate Calories per 100 grams	500.	450.	400.	400.	400.

* Vitamin B Prep. ("Vitavose") is a vitamin B-rich product manufactured by the E. R. Squibb Company of New York. Experiments in the Department of Physiological Chemistry, Yale University, have demonstrated that dogs, on rations similar to these used in our experiments, need about 0.6 grams per kilogram per day to satisfy their vitamin B requirement.

** The commercial meat residue used was obtained from the Valentine Meat Juice Company, Richmond, Virginia. It contains approximately 13 per cent nitrogen and 10 per cent fat.

*** The salt mixture used in the rations had the following composition:

	Per cent by Weight
Sodium chloride	51.0
Calcium lactate	20.4
Magnesium citrate	20.4
Ferric citrate	5.1
Potassium iodide	1.0
Potassium chloride	2.0

TABLE VI

EFFECT OF COCOANUT OIL ON NITROGEN BALANCE IN THE DOG

How Administered	Experimental Period	Duration of Period	No. of Dog	No. of Ration	Average Daily Nitrogen Output	Average Daily Nitrogen Intake	Average Daily Nitrogen Balance	General Effect of Treatment	Total Food Intake
		days			grams per kg.				Calories per kg.
1. Subcutaneous Injections	Pre-inj.	7	4	2	.15	.07	−0.08	Practic-	44.1
	Injections	7			.16	.07	−0.09	ally no	45.1
	post-inj.	7			.16	.07	−0.09	change	45.5
2. Intraperitoneal Injections	a. Pre-inj.	7	6	3	.19	.09	−0.10	Slight	34.7
	Injections	7			.18	.10	−0.08	sparing	35.5
	Post-inj.	7			—	—	—	effect	37.9
	b. Pre-inj.	7	6	5	.46	.34	−0.12	Definite	57.8
	Injections	5			.40	.35	−0.05	sparing	58.8
	Post-inj.	12			.40	.36	−0.04	effect	60.4
	c. Pre-inj.	7	4	2	.17	.07	−0.10	Slight	47.4
	Injections	7			.16	.08	−0.08	sparing	49.1
	Post-inj.	7			.16	.08	−0.07	effect	49.0
3. Orally 10 grams daily	Pre-feeding	7	6	3	.20	.11	−0.09	Practic-	43.5
	Feeding	7			.21	.11	−0.10	ally no	45.6*
	Post-feeding	—			—	—	—	change	—

* Does not include extra oil that was mixed with the ration.

TABLE VII

EFFECT OF PEANUT OIL ON NITROGEN BALANCE IN THE DOG

How Administered	Experimental Period	Duration of Period	No. of Dog	No. of Ration	Average Daily Nitrogen Output	Average Daily Nitrogen Intake	Average Daily Nitrogen Balance	General Effect of Treatment	Total Food Intake
		days				grams per kg.			Calories per kg.
4. Subcutaneous Injections	Pre-inj.	7	2	2	.11	.06	−0.5	Slight	39.3
	Injections	7			.10	.07	−0.3	sparing	40.4
	Post-Inj.	7			.10	.07	−0.3	effect	40.5
5. Intraperitoneal Injections	a. Pre-inj.	7	2	2	.10	.06	−0.04	Almost	41.0
	Injections	7			.09	.06	−0.03	no	41.2
	Post-inj.	7			.09	.06	−0.03	changes	41.2
	b. Pre-inj.	6	2	3	.11	.10	−0.01	No	42.9
	Injections	7			.12	.11	−0.01	change	43.2
	Post-inj.	7			.12	.11	−0.01		44.0
	c. Pre-inj.	7	3	4	.35	.54	+0.19	Definite	82.0
	Injections	7			.36	.48	+0.12	decrease	81.2
	Post-inj.	–			—	—	—		—
6. Orally 20 grams daily	Pre-feeding	7	5	3	.17	.14	−0.03	Almost	51.5
	Feeding	7			.18	.15	−0.03	no	52.0*
	Post-feeding	9			.19	.15	−0.04	change	52.5

* Does not include the extra oil that was mixed with the ration.

TABLE VIII

EFFECT OF BUTTER OIL ON NITROGEN BALANCE IN THE DOG

How Administered	Experimental Period	Duration of Period	No. of Dog	No. of Ration	Average Daily Nitrogen Output	Average Daily Nitrogen Intake	Average Daily Nitrogen Balance	General Effect of Treatment	Total Food Intake
		days			grams per kg.				Calories per kg.
7. Intraperitoneal Injections	a. Pre-inj.	8	5	5	.25	.38	+0.13	Almost	63.8
	Injections	4			.27	.39	+0.12	no	65.8
	Post-inj.	–			—	—	—	change	
	b. Pre-inj.	7	3	2	.17	.09	−0.08	Almost	56.3
	Injections	7			.17	.09	−0.08	no	57.5
	Post-inj.	7			.15	.09	−0.06	change	59.6
	c. Pre-inj.	6	3	4	.31	.56	+0.25	Definite	83.7
	Injections	7			.36	.57	+0.21	decrease	85.1
	Post-inj.	7			.35	.54	+0.19		82.0
8. Orally 10 grams daily	Pre-feeding	7	3	5	.33	.39	+0.06		72.3
	Feeding	7			.39	.39	0.0	Decrease	72.8*
	Post-feeding	–			—	—	—		

* Does not include the extra oil that was mixed with the ration.

TABLE IX

RECORD OF FOOD AND NITROGEN INTAKE OF DOGS

	Ration used	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6
Average food intake (grams per kg.)	1	11.6–13.4	13.0–13.4	11.6	—	—
	2	9.8–10.3	13.6–15.0	11.0–12.2	10.7–11.4	—
	3	10.7–11.0	—	15.1–15.5	11.7–14.7	8.7–11.4
	4	—	19.9–21.2	—	—	—
	5	—	18.0–18.2	—	15.3–16.7	13.6–15.0
Average Caloric Intake (Calories per kg.)	1	57–66	64–66	57–62	—	—
	2	39–41	56–60	44–49	49–51	—
	3	41–44	—	60–63	48–53	34–46
	4	—	79–85	—	—	—
	5	—	72–73	—	63–67	54–61
Average Nitrogen Intake (grams per kg.)	1	0.08	0.07–0.09	0.08	—	—
	2	0.06–0.07	0.08–0.09	0.07–0.08	0.07	—
	3	0.10–0.11	—	?	0.11–0.15	0.09–0.11
	4	—	0.48–0.57	—	—	—
	5	—	0.39	—	0.37–0.39	0.32–0.36
General Nitrogen Balance	1	negative	negative	negative	—	—
	2	negative	negative	negative	negative	—
	3	negative	—	negative	negative	negative
	4	—	positive	—	—	—
	5	—	positive	—	positive	negative, except for second week

Protocols to Chart VIII

Dog 2

Injection of peanut oil in period 4 subcutaneously and in periods 5a and 5b intraperitoneally. (See Table VII.)

On autopsy apparently all the peanut oil that had been injected intraperitoneally was still present in the abdominal cavity. There was a large amount of clear free oil in the open cavity and the intestines and other organs were covered with a large amount of white fat, in spite of steady losses in weight, low caloric intake, and constant negative nitrogen balances (Table IX). There was no edema. The abdominal organs were very pale.

There was still considerable clear oil in the subcutaneous areas around the neck into which the oil had been injected three months before autopsy.

Dog 3

Injection of peanut oil intraperitoneally in period 5c, (Table VII), and of butter oil intraperitoneally in periods 7b and 7c (Table VIII) with its oral administration in period 8 (Table VIII).

On autopsy there was found a considerable amount of free yellow oil in the abdominal cavity

which was apparently a mixture of butter oil and peanut oil. There was no body or organ fat present and the animal was very emaciated.

While the animal was on ration 2, two 10 cubic centimeter injections of butter oil were given under the loose skin of the neck. When autopsied five or more months later none of this could be detected in this region. It had apparently been metabolized.

Dog 4

Injection of cocoanut oil subcutaneously in period 1 and intraperitoneally in period 2c (Table VI).

When the animal was autopsied there was no trace of the cocoanut oil that had been injected subcutaneously seven and a half months earlier. There was considerable free oil in the abdomen, however. This animal was injured during an injection immediately after period 2c. Acute illness and peritonitis resulted with the formation of such a tough white coating over all the viscera that absorption of foreign material in the abdominal cavity would have been almost impossible. There was very severe edema and marked emaciation.

Dog 5

Oral administration of peanut oil (period 6, Table VII), and intraperitoneal injection of butter oil (period 7a).

The animal had received intraperitoneal injections of cod liver oil shortly after being placed on ration 3, with resultant severe illness. When autopsied many weeks later, none of the deep yellow fatty deposits, always observed in rats after such treatment, could be seen around the abdominal organs. There was a large amount of clear yellow oil in the abdominal cavity, but it did not have the odor of cod liver oil and was evidently the butter oil that had been injected a week before the animal was killed.

Dog 6

Intraperitoneal injections of cocoanut oil,—periods 2a and 2b and its oral administration in period 3 (Table VIII).

There was a large amount of free colorless oil in the abdominal cavity when the animal was autopsied one week after the last intraperitoneal injection. There was no edema. No body fat or organ fat was visible.

General Summary

Cod Liver Oil Injections.—Both vitamins A and D in cod liver oil can be utilized by young rats when the oil is administered parenterally. That the oil when so given has toxic properties is evident from the marked deterioration of the physical condition of all animals used, and from the definite increases in nitrogen losses resulting in the dog. The body cells treat the injected oil or its emulsion as they would any irritating foreign substance,—through the protective reticulo-endothelial system. Not all of the oil administered parenterally to rats could be recovered by the extraction method used,—whether the animals were killed in a comparatively good state of nutrition or died of starvation. Some of the oil may have been transported by way of the lymph and excreted in urine or feces or it may have been used as a source of energy; or the extraction method may have been inadequate. The evidence seems generally to indicate that this oil is probably of little or no value nutritively when so

given. There was always an abundance of deep yellow encapsulated cod liver oil present in the bodies of the rats at death. Sudan III separates from cod liver oil in which it is dissolved very soon after the mixture is injected. The dye is rapidly excreted by way of the sebaceous glands and the oil particles are promptly engulfed by the endothelial or giant cells and stored in this encapsulated form in the lymphatic system.

Butter Oil Injections.—Unquestionably young rats deprived of vitamin A receive protection against the effects of this dietary deficiency or can be cured of existing symptoms by the parenteral administration of butter oil. None of the animals used showed any local or general physical reaction to the injected oil. In most of the autopsies of animals that had received intraperitoneal injections of butter oil there was considerable free oil present at death, with only a relatively small proportion encapsulated. It is apparently only mildly irritating. When Sudan III is dissolved in the oil before injection into adult rats, the dye remains in solution and is only very gradually excreted by way of the sebaceous glands. In these animals free oil could always be recovered from the body after death and this oil had a deep red color, while the encapsulated oil was orange in color. Very little butter oil could be extracted from the bodies of the young rats used in series A experiments, but there was little or no evidence of its utilization by adult rats in the experiments of series C and D. Starvation seemed to have little influence in forcing the utilization of butter oil in mature animals. Butter oil, in the amounts used and under the experimental conditions described, had no effect as a nitrogen-sparing agent. All of the oil injected subcutaneously into the dog had disappeared however, before autopsy, which indicates that perhaps it has been gradually metabolized.

Cocoanut Oil Injections.—Practically all the cocoanut oil that was injected into adult rats in series D experiments in which the animals were subsequently starved, had disappeared by the time the animals were autopsied. If, however, the rats were kept in a good state of nutrition following the injections a great deal of oil could be recovered (Series C experiments). Sudan III remained in solution in this oil as long as the oil remained in the body. As in the other experiments this dye is finally excreted through the sebaceous glands. Cocoanut oil injections never caused any local or general reaction in the animals used. This oil is the only oil experimented with which, on parenteral administration, seemed to have a definite though slight sparing effect on protein catabolism. Furthermore, the oil given subcutaneously to dogs had completely disappeared by the time the animals were autopsied.

Peanut Oil Injections.—That the adult rats may be able to utilize some of the peanut oil administered parenterally is indicated by experiments in Series C, D, and E. It seems to make little difference in the utilization of this oil whether the animals are starved or not after its injection. Sudan III behaves, when administered with this oil, just as it does when given in solution in cocoanut oil. All evidence seems to indicate that the dog is probably unable to make any nutritive use of peanut oil administered parenterally.

Conclusions

With the possible exception of cocoanut oil under conditions of nutritive emergency the investigated oils introduced parenterally are too slowly metabolized, if at all, to be a dependable source of energy to animals.

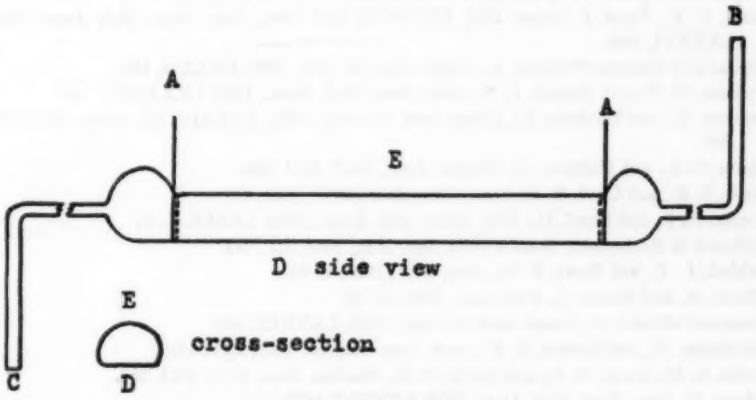


Fig. 1.—Quartz Sterilizing Tube

A–A. Wire attachments to ends of mercury tubes.
B. Inlet tube for oil.
C. Outlet tube for oil.
B and *C* about 1/8 inch bore and extending beyond hood of lamp.
D. Flat bottom of tube, about 3/4 inch wide.
E. Curved top of tube.
Inside diameter *E* to *D* about 1/2 inch.

The utilization or non-utilization of fat-soluble vitamins A and D and Sudan III present in an oil at the time of its injection, cannot be regarded as proof of the utilization or non-utilization of the oil which held it in solution.

Fats administered parenterally are generally too slowly metabolized

to exert a sufficient influence on nitrogen catabolism to make this method of evaluation practicable.

Further experiments should be conducted on rats to study the effect of high-protein, low-calorie diets on the utilization of injected fats, and the effect likewise of other measures designed to increase the rate of metabolism of experimental animals.

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CALCIUM AND PHOSPHORUS METABOLISM IN DAIRY COWS III. THE ADEQUATE RATION FOR HIGH-PRODUCING COWS AND THE EFFECT OF EXERCISE ON CALCIUM, PHOSPHORUS AND NITROGEN BALANCES*

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INTRODUCTION

IN A former paper (1) it was shown that the assimilation of the calcium of alfalfa hay by dairy cows was much less when the alfalfa was exposed, in curing, to the sun and rain than when it was cured under cover without such exposure. Later evidence (2) showed the importance of the calcium-phosphorus ratio in the feed and indicated that the optimum value for this ratio is between 1.0 and 1.5. Experiments carried on at the Dairy Experiment Station of the Bureau of Dairy Industry, at Beltsville, Md. and at other stations have shown that, generally speaking, the calcium of natural feeds is assimilated to a greater extent than the calcium of minerals. For example, at the Wisconsin Experiment Station [(1) p. 850] the calcium assimilation from a lawn-grass ration was found to be 33.9 per cent and that from a lawn grass and bone meal ration was 18.3 per cent. Later results by the Wisconsin investigators (3) showed a calcium assimilation from a lawn grass and marl ration of 26 per cent where the cows were giving 45–60 pounds of milk per day.

In order to test the validity of these views and particularly to determine whether a ration in accordance with them could be devised which would maintain high-producing cows in calcium and phosphorus equilibrium, the present experiment was undertaken. It was considered of interest also to learn the effect of exercise on mineral and nitrogen balances. Cows, in general, are accustomed to some exercise; but the usual method of conducting balance experiments allows little. During the last three weeks of this experiment the cows were given thirty minutes of exercise per day by walking either out-of-doors or in the barn, according to the weather.

* The first two papers of this series appeared in the Journal of Agricultural Research, XXXII, 833 (1926) and XXXV, 625 (1927).

Experimental

Two pure bred, 6 year-old, Holstein cows giving 28 and 22 kgs. of milk daily were selected for the experiment. They were placed in the metabolism stalls two weeks prior to the beginning of the experiment in order that they might become accustomed to the confinement and that their optimum feed consumption might be determined. They were given a ration composed of well-cured alfalfa hay, mangel beets, and a grain mixture made up as follows: Wheat bran, 30 per cent; corn meal, 40; soy bean meal, 20; linseed meal, 10; sodium chloride, 1; and disodium phosphate, 5. The alfalfa hay was a second cutting, grown at Beltsville. It was brought into the barn the day after cutting, having received little exposure to the sun since the weather was cloudy. It was spread on the barn floor where it remained for ten days with frequent turnings. The hay inspector graded it U. S. No. 1 Alfalfa, 43 per cent leafiness and 65 per cent green color. It was chopped and stored in sacks. Sodium phosphate was used in the grain mixture in order to maintain a satisfactory ratio between calcium and phosphorus.

The experimental routine was similar to that pursued in former work at this station (1, 2).

The cows were placed on the experiment about six weeks after parturition. They were fed twice a day and as much as they would clean up. During the first four weeks they received about 95 per cent of their energy requirements, according to the Savage standard; and during the last three weeks, about 105 per cent. Throughout the experiment they received an excess of protein ranging from 115 to 140 per cent according to the Savage standard. In the work at the Beltsville station it has been difficult to induce high-producing cows to consume their energy requirements, according to this standard, in the early weeks of lactation. The cows drank water *ad libitum*, 50–70 kgs. per day, and were milked three times a day. The cows were not bred during the experiment. When exercised the cows were accompanied by an attendant with a dipper to collect any excreta. With observance of proper precautions no trouble was experienced. The cows were exposed to sunlight during only a part of the exercise periods and probably received little ultra-violet light as the experiment was conducted in late fall.

The analytical methods include a modification of the McCrudden method for calcium, the gravimetric method for phosphorus, and the Kjeldahl method for nitrogen.

The cows gained in weight during the experiment, but cow 205 appar-

ently lost some of her gain during the exercise period. They were weighed on each of three successive days at the beginning of each month. Table I records these weights.

Table II shows the composition of the different batches of feed used during the course of the experiment, Table III gives the quantity of feed

TABLE I
BODY WEIGHTS OF THE TWO COWS USED IN THE EXPERIMENT

Cow	November				December				January			
	1	2	3	Av.	1	2	3	Av.	1	2	3	Av.
	kgs.	kgs.	kgs.	kgs.	kgs.	kgs.	kgs.	kgs.	kgs.	kgs.	kgs.	kgs.
205	485	499	488	491	491	496	499	495	485	491	499	492
206	613	616	615	615	620	617	619	619	617	629	632	626

TABLE II
COMPOSITION OF FEEDS USED IN THE EXPERIMENT

Period	Feed	H_2O	Ca	P	N
		%	%	%	%
Nov. 3–Dec. 9	Alfalfa Hay I	12.42	1.412	0.252	2.52
Dec. 10–21	Alfalfa hay II	12.64	1.423	0.238	2.50
Nov. 3–Dec. 21	Mangel beets	88.51	0.019	0.020	0.17
Nov. 3–15	Grain I	13.94	0.102	1.091	3.14
Nov. 16–27	Grain II	14.09	0.106	1.106	3.14
Nov. 28–Dec. 9	Grain III	13.96	0.107	1.113	3.04
Dec. 10–20	Grain IV	13.66	0.104	1.107	3.10
Dec. 21	Grain V	14.60	0.106	1.104	3.16

TABLE III
QUANTITY OF FEEDS CONSUMED BY THE TWO COWS USED IN THE EXPERIMENT

Week	Cow 205				Cow 206			
	Hay Offered	Beets Offered	Grain Offered	Feed Refused	Hay Offered	Beets Offered	Grain Offered	Feed Refused
	Kgs.	Kgs.	Kgs.	Kgs.	Kgs.	Kgs.	Kgs.	Kgs.
Nov. 3–9	49	126	56	none	56	126	56	0.4 hay
Nov. 10–16	56	126	56	none	56	126	56	none
Nov. 17–23	56	126	60.2	none	56	126	56	none
Nov. 24–30	60.2	126	60.2	none	56	126	56	none
Dec. 1–7	63	126	63	none	56	126	56	none
Dec. 8–14	63	126	67.2	none	56	126	60.2	none
Dec. 15–21	63	126	55.8	none	56	126	60.2	none

consumed, and Table IV the composition of the milk. In Tables V, VI, and VII are shown the calcium, phosphorus, and nitrogen balances and assimilations, the milk yields, Ca/P ratios, and energy intakes for the two animals.

TABLE IV

COMPOSITION OF MILK FROM THE TWO COWS USED IN THE EXPERIMENT

Week	Cow 205			Cow 206		
	Ca %	P %	N %	Ca %	P %	N %
Nov. 5–11	0.108	0.092	0.475	0.122	0.091	0.541
Nov. 12–18	0.111	0.093	0.492	0.122	0.092	0.526
Nov. 19–25	0.109	0.094	0.489	0.117	0.094	0.522
Nov. 26–Dec. 2	0.114	0.094	0.501	0.118	0.092	0.530
Dec. 3–9	0.113	0.094	0.508	0.120	0.089	0.524
Dec. 10–16	0.113	0.097	0.526	0.117	0.090	0.529
Dec. 17–23	0.116	0.096	0.501	0.122	0.092	0.525

The term "assimilation" used in these tables, referring to the total intake of elements minus the outgo in urine and feces, is a little misleading in the case of nitrogen, since a considerable amount of metabolic nitrogen occurs in the urine and feces. The term is believed to be of value, however, if the sense in which it is used is kept in mind.

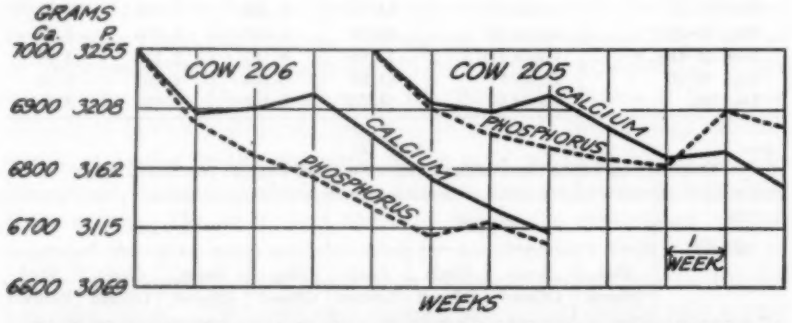

FIG. 1. Changes in calcium and phosphorus content of the bodies of cows 205 and 206 throughout the course of the experiment. The solid line represents the calcium and the broken line the phosphorus.

In Figure 1 are shown graphically the changes in calcium and phosphorus content of the bodies of the two cows. The assumption is made that at the beginning of the experiment the cows had in their bones

TABLE V

DAILY CALCIUM BALANCE, ASSIMILATION, MILK YIELD AND Ca/P INTAKE

Week	Ca in urine and feces	Ca in milk	Total Ca excreted	Ca intake	Ca balance	Ca assimilation		Ca/P in intake	Milk Yield
	gm.	gm.	gm.	gm.	gm.	gm.	% intake		kg.
Cow 205									
Non-exercise period									
Nov. 5–11	91.9	31.1	123.0	110.5	−12.5	18.6	16.8	1.02	28.8
12–18	95.6	30.8	126.5	124.7	− 1.8	29.0	23.3	1.12	27.8
19–25	91.5	30.1	121.6	125.5	+ 4.0	34.0	27.1	1.06	27.6
26–Dec. 2	110.4	31.7	142.1	134.1	− 8.0	23.7	17.7	1.11	27.8
Average	97.4	30.9	128.3	123.7	− 4.6	26.3	21.2	1.08	28.0
Exercise period									
Dec. 3–9	116.1	31.0	147.1	140.2	− 6.9	24.1	17.2	1.11	27.4
10–16	109.9	30.2	140.1	141.3	+ 1.2	31.4	22.2	1.07	26.7
17–23	118.9	28.9	147.9	139.9	− 8.0	20.9	15.0	1.23	24.9
Average	115.0	30.0	145.0	140.5	− 4.6	25.5	18.1	1.14	26.3
Cow 206									
Non-exercise period									
Nov. 5–11	110.4	27.9	138.3	123.8	−14.5	13.4	10.8	1.12	22.9
12–18	96.5	27.2	123.7	124.7	+ 0.9	28.2	22.6	1.12	22.3
19–25	94.4	27.0	121.4	124.9	+ 3.5	30.5	24.4	1.11	23.1
26–Dec. 2	109.6	25.9	135.6	125.0	−10.6	15.3	12.3	1.11	22.0
Average	102.7	27.0	129.8	124.6	− 5.2	21.9	17.5	1.11	22.6
Exercise period									
Dec. 3–9	108.7	25.7	134.5	125.0	− 9.5	16.2	13.0	1.11	21.5
10–16	107.6	25.5	133.1	126.0	− 7.1	18.4	14.6	1.06	21.8
17–23	106.0	26.4	132.4	126.3	− 6.1	20.3	16.1	1.07	21.6
Average	107.4	25.9	133.3	125.8	− 7.6	18.3	14.6	1.08	21.6

7000 gms. calcium and 3255 gms. phosphorus. The amounts of calcium and phosphorus assumed to be present in their bodies at the beginning of the experiment will not, however, affect the form of the graph. From the figure it is evident that cow 205 lost about 3 per cent of the calcium and 2 per cent of the phosphorus, and cow 206 lost about 4 per cent of the calcium and 5 per cent of the phosphorus of their bodies during the seven weeks of the experiment. This rate of loss, if continued for any length of time, might lead to a considerable impoverishment of the mineral resources of the cows. The situation would be worse with cows not so well fed.

Discussion

The results of this experiment were somewhat disappointing. The conditions were thought to be ideal. The cows appeared contented and as comfortable as could be expected with the necessary confinement. They received what was considered an excellent ration. Although the energy intake was somewhat below the Savage standard in the early part of the experiment, the fact that they showed no loss in body weight and sustained their milk yields would seem to indicate no shortage of energy. The intake of digestible protein was above the Savage standard in all periods (15–40%); the intakes of calcium and phosphorus were high (calcium, 120–140 gms. daily, and phosphorus, 110–125 gms. daily). The ratio of intake of Ca to intake of P ranged from 1.09 to 1.19. The alfalfa was cured in such a manner as to preserve, as nearly as possible, the natural state of the mineral and nitrogen supplies and the vitamins. Recently harvested mangel beets might reasonably be expected to augment the supply of vitamins.

In spite of these apparently favorable circumstances the cows remained, on the whole, in negative calcium and phosphorus balance throughout the entire experiment. It seems that high-producing cows have so great an impulse to secrete milk that it is difficult for them to assimilate sufficient calcium and phosphorus to satisfy the demand for these elements. Forbes (4) first called attention to this condition, and modern knowledge of nutrition is apparently still unable to cope with it. It is believed, however, that the quality of the feeds used in this experiment has improved the situation. The negativity of these balances is not great when compared with the results obtained in many other experiments conducted at this station, even though the milk yield is much greater. No known comparable results have been obtained at other stations. It is believed

TABLE VI

DAILY PHOSPHORUS BALANCE AND ASSIMILATION

Week	P in urine and feces	P in milk	Total P excreted	P intake	P balance	P assimilation	
	gm.	gm.	gm.	gm.	gm.	gm.	% intake
Cow 205							
Non-exercise period							
Nov. 5–11	88.6	26.5	115.0	108.6	−6.4	20.0	18.4
12–18	88.4	25.8	114.2	111.3	−2.8	23.0	20.6
19–25	94.8	25.9	120.7	118.9	−1.8	24.1	20.3
26–Dec. 2	95.8	26.1	122.0	120.7	−1.2	24.9	20.6
Average	91.9	26.1	118.0	114.9	−3.1	23.0	20.0
Exercise period							
Dec. 3–9	101.4	25.8	127.1	126.5	−0.6	25.2	19.9
10–16	99.9	25.9	125.8	131.8	+6.0	32.0	24.3
17–23	91.0	23.9	115.0	113.3	−1.7	22.3	19.6
Average	97.4	25.2	122.6	123.9	+1.2	26.5	21.3
Cow 206							
Non-exercise period							
Nov. 5–11	98.3	20.8	119.2	111.0	−8.2	12.6	11.4
12–18	94.4	20.5	115.0	111.3	−3.6	16.9	15.2
19–25	93.0	21.7	114.7	112.3	−2.4	19.3	17.2
26–Dec. 2	95.8	20.2	116.0	112.6	−3.4	16.8	14.9
Average	95.4	20.8	116.2	111.8	−4.4	16.4	14.7
Exercise period							
Dec. 3–9	97.1	19.1	116.2	112.9	−3.3	15.8	14.0
10–16	97.4	19.6	117.1	118.4	+1.4	21.0	17.7
17–23	100.3	19.9	120.3	117.8	−2.4	17.5	14.9
Average	98.3	19.5	117.9	116.4	−1.4	18.1	15.5

that if the precautions observed in this experiment with regard to feeds were generally practiced, better dairy cow nutrition would result. The problem of maintaining high-producing dairy cows in calcium and phosphorus equilibrium, however, is still unsolved.

The effect of exercise on the mineral balances appears to be rather uncertain and probably not very significant. Negative calcium balances continued in spite of larger calcium intakes and the assimilations were less than in the non-exercise periods. The increased phosphorus intakes in the exercise periods were accompanied by improved phosphorus balances and assimilations.

The effect of exercise on the nitrogen metabolism, on the other hand, was very definite. Strongly positive nitrogen balances were changed to negative ones, assimilations were distinctly less, and elimination of nitrogen in the urine and feces was increased.

It is not known whether sufficient energy was provided to cover the added demands of exercise. It seems, however, that the energy provision should have been adequate since the cows received 5 per cent in excess of the Savage standard (a standard usually regarded as liberal) and the exercise which they took was light. When they were exercised outdoors the weather was sometimes fairly cold (about 20 degrees F) and windy. The effect of this, however, is difficult to determine.

At any rate a destruction of body protein occurred which, assuming the energy supply to be adequate, must be regarded as the result of exercise. This fact lends support to the contention of those investigators (5) who claim that although carbohydrate is the chief source of muscular activity, a small amount of nitrogen is also needed in exercise. This appears to be the case; otherwise no destruction of body protein would have been observed because the protein of the feed was ample to cover maintenance and milk production.

Summary

Two cows, receiving an excellent dry ration consisting of well-cured alfalfa hay, mangel beets, and a good grain mixture (Ca/P ratio 1.09–1.19), and yielding 27 and 22 kgs. of milk daily, remained in prevailingly negative calcium and phosphorus balances throughout a period of seven weeks. This occurred in the early part of the lactation period.

During the last three weeks of the experiment the introduction daily of one-half hour's exercise produced slight and probably insignificant effects on the calcium and phosphorus metabolism but a very marked effect on the

TABLE VII

DAILY NITROGEN BALANCE AND ASSIMILATION

Week	N in urine and feces	N in milk	Total N excreted	N intake	N balance	N assimilation		% of nutr. req. (Savage)	
	gm.	gm.	gm.	gm.	gm.	gm.	% intake	D.P.	T.D.N.
Cow 205									
Non-exercise period									
Nov. 5–11	319.2	136.6	455.8	458.0	+ 2.2	138.8	30.3	106.6	85.1
12–18	330.6	136.6	467.1	483.2	+16.1	152.6	31.6	115.3	91.5
19–25	340.3	134.9	475.2	502.1	+26.9	161.8	32.2	120.8	95.4
26–Dec. 2	375.4	139.3	514.7	512.9	− 1.8	137.5	26.8	123.1	97.2
Average	341.4	136.9	478.2	489.1	+10.9	147.7	30.2	116.4	92.3
Exercise period									
Dec. 3–9	393.9	139.3	533.2	530.8	− 2.3	136.9	25.8	132.2	104.4
10–16	404.1	140.7	544.8	552.3	+ 7.5	148.2	26.8	140.1	110.0
17–23	396.0	125.0	521.0	503.2	−17.8	107.2	21.3	133.7	104.7
Average	398.0	135.0	533.0	528.8	− 4.2	130.8	24.6	135.4	106.4
Cow 206									
Non-exercise period									
Nov. 5–11	335.1	123.7	458.9	481.8	+22.9	146.6	30.4	128.5	96.3
12–18	341.7	117.4	459.1	483.2	+24.1	141.5	29.3	131.3	98.0
19–25	328.5	120.5	449.0	483.2	+34.2	154.7	32.0	128.0	96.0
26–Dec. 2	356.1	116.4	472.5	479.2	+ 6.7	123.1	25.7	133.0	99.0
Average	340.4	119.5	459.9	481.9	+22.0	141.5	29.4	130.2	97.3
Exercise period									
Dec. 3–9	367.1	112.4	479.5	475.2	− 4.3	108.1	22.8	135.9	101.0
10–16	379.6	115.4	495.0	496.5	+ 1.5	116.9	23.5	139.9	103.8
17–23	393.5	113.6	507.1	498.1	− 9.0	104.7	21.0	140.7	104.3
Average	380.1	113.8	493.9	489.9	− 3.9	109.9	22.4	138.9	103.0

nitrogen metabolism. Without allowing for exercise, the animals received 5 per cent more than their energy requirements calculated on the basis of the Savage standard.

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THE EFFECT OF HEAT ON THE ANTINEURITIC VITAMIN OF MILK

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IN STUDIES pertaining to the nutritive value of heat treated milk Hartwell (1) has shown that the superheating involved in the process of evaporating milk results in considerable destruction of the antineuritic vitamin. These findings have been confirmed by Daniels and Brooks (2) with both rats and pigeons. The method used in the tests with rats consisted in comparing the growth and behavior of suckling young whose mothers, during the lactation period, were receiving high protein rations in which the antineuritic vitamin was furnished by given amounts of fresh, quickly boiled, and evaporated milk, respectively. When adequate amounts of the antineuritic vitamin are present the animals develop normally. Evidence of too little is made manifest in the young by a failure to gain or a sudden loss in weight, muscle incoordination, convulsions, frequently accompanied by unusual outcries, and subsequent death, usually between the thirteenth and sixteenth days. That death in these cases was not caused by too little food is indicated by the fact that clotted milk was often present in the stomachs of the dead animals. The most frequent and outstanding pathological findings have been cerebral hemorrhages, particularly noticeable at the junction of the occipital and parietal bones.

Reports of other investigations pertaining to the destruction of the antineuritic vitamin in superheated milk do not corroborate these findings. Dutcher (3) was unable to obtain definite evidence of Vitamin B destruction in milk evaporated by the vacuum and aeration processes. However, when these milks were fed at very low levels there was a suggestion of vitamin destruction. The author states that possibly aeration, oxidation, or heat brought about some slight destruction of Vitamin B. Gibson and Concepcion (4) also observed that milk loses very little, if any, of its antineuritic value as the result of autoclaving for two hours at 125°C. Both investigators worked with older animals. It is probable that the rapidly growing suckling young are much more sensitive to a deficiency of the antineuritic vitamin than are more mature animals.

Evaporated milk is not used widely in the artificial feeding of infants, although there are certain pediatricians who advocate its use. There are, however, other forms of heat treated milk, for example dried milk and various canned-milk preparations as well as boiled and pasteurized milk, which are being used extensively in infant nutrition. It seemed probable that some of these, also, may be low in the antineuritic vitamin. To determine this we have studied the behavior of suckling rats whose mothers were receiving high protein rations in which the particular milk or milk preparation in question was substituted for the evaporated milk in the Hartwell ration. The investigation has included three types of dried milk: one, made by the spray process; a second, prepared by passing milk over superheated rollers; and a desiccated albumin milk. We have made tests, also, with commercially pasteurized and home pasteurized milk, as well as with quickly boiled milk cooled slowly in large quantities, and rapidly in small amounts. Milk from two dairies has been investigated. Tests with evaporated milk have been repeated, and in some of these larger amounts of the evaporated milk have been used. In order to make sure that the results obtained with the heated and superheated products were due to the destruction of the antineuritic vitamin we have fed other groups of animals similar rations to which have been added substances known to contain the vitamin in question. These have included yeast, an autolyzed yeast product, and an alcoholic extract of wheat embryo. In some cases these were superheated, thus ruling out the possibility of misinterpreting the stimulating effect of other substances.

The superheated yeast and yeast product used in the rations were prepared by autoclaving at fifteen pounds pressure for five hours. Before autoclaving, the starch-free yeast* was air dried and pulverized; the autolyzed yeast was dissolved in distilled water. The wheat-embryo preparation was an 80% alcoholic extraction so diluted that 1 cc. contained the vitamin B from one gram of wheat germ.

The process used in the commercial pasteurized milk (Dairy No. 1) consisted in holding the milk at 145°F for thirty minutes in an open glass-lined tank. During this time the milk was kept continuously in motion. It was then cooled quickly by passing over a series of refrigerator pipes. The home pasteurized milk (Closed Method A) was prepared by heating milk in closed one quart glass bottles to 145° F in a water bath. The milk was held at this temperature for thirty minutes, when it was cooled in running water. In the open method (Method B) the milk was

* We are indebted to the Fleischmann Co. for the starch-free yeast.

heated in an open aluminum sauce pan on the water bath, care being taken to keep the milk in motion during the process. The temperature and time of heating were the same as in the other methods. This procedure did not duplicate the commercial process, but it allowed for more exposure to air than pasteurization by Method A.

The quickly boiled milk was prepared in three ways: 1.—The milk in one-pint lots was brought quickly to the boiling point in an open pan; this was then cooled as quickly as possible in running water, the entire process taking approximately fifteen minutes. 2.—The milk was heated quickly in one pint lots, and then poured in a pail which held three quarts. This was continued until the pail was full. It was then allowed to stand at room temperature about one hour, when it was put into the refrigerator. Repeated tests showed that during the first hour after heating the temperature of the milk remained between 100° C. and 62.5° C. During the second hour the temperature dropped to 32.5° C. 3.—100 cc. of milk were brought quickly to the boiling temperature in an open saucepan, and allowed to cool quickly to room temperature.

In the investigation we have followed, in general, Hartwell's (5) method. The composition of the milk preparations tested have differed somewhat. Therefore it was necessary to make slight modifications, in order to obtain rations having the same nutritive ratio (2.89). For example, in the ration made with the milk dried by the roller process, because of the lower fat content, 4.5 grams of cornstarch were added. This was extracted 48 hours with 80 per cent alcohol.

In all cases the mother animals were fed the stock ration until just previous to or just following parturition, when they were given the experimental ration. When the litters were twenty-four hours old the number was reduced to four in some groups and to six in others. From the fourth day these were weighed regularly every four days.

The relative antineuritic vitamin content of the foods in question is shown by the growth and behavior of the suckling young during a period of twenty-four days. According to Evans and Burr (6) a young rat which weighs 6 grams at birth and 40 grams at twenty-one days of age is normal. This allows for an average gain of approximately 12 grams per week. Such rats whose mothers are receiving adequate diets never manifest the peculiar symptoms of the sucklings on the Hartwell ration. In our investigation we have recorded the average gain per rat per week up to sixteen days and between sixteen and twenty-four days. In a number of cases the percentage of weight gain or loss of the mother animals either

at the time of the death of the young or at the end of the nursing period (21 days) is recorded. The results of the investigation are summarized in the following tables.

Results

A comparison of the growth curves of the suckling young of mothers receiving rations which depend largely for their antineuritic vitamin content on superheated milks with those of the young of mothers receiving similar rations made with equivalent amounts of quickly boiled milk, or rations of superheated milk supplemented with the autolyzed yeast preparation, wheat embryo extract, or yeast, leaves no doubt that some of these superheated milks are low in the antineuritic vitamin. This is made manifest not only by the less satisfactory growth performance of the young, but by the convulsions which under the conditions of the experiment are characteristic of the deficiency. Of the superheated milks tested, that made by the roller process was the only one in which there was little indication of a considerable vitamin destruction. The results with this were quite comparable to those with milk which was boiled in 100 cc. lots and cooled quickly.

The three samples of commercial pasteurized milk were also found to be lower in the antineuritic vitamin than was the milk which was quickly boiled, or pasteurized by the hold method in closed one-quart bottles (Method A). All tests were made in May, June, July, and December, with milk from two different dairies. The results with each were fairly comparable. A comparison of the behavior of the animals receiving the rations made with milk boiled and cooled rapidly in small amounts, suggests that there was some destruction of the vitamin in the slowly cooled product.

In an attempt to obtain a measure of the degree of destruction of the vitamin in evaporated milk we have fed lactating animals the Hartwell ration made with larger amounts of evaporated milk. In these the quantity of evaporated milk in each ration differed by 5 cc. until a level of 75 cc. was reached. In each ration the casein and bread were correspondingly decreased in order to maintain as far as possible the same nutrition ratio. Slightly more fat and inorganic salts were contained in the higher milk mixtures. The growth curves of the young were considerably better on the higher levels of milk, and the convulsions were delayed. At the 50 cc. level these were observed between the thirteenth and fifteenth days, whereas at the 75 cc. level they were first noted on the nineteenth day.

TABLE I

THE DEVELOPMENT OF SUCKLING YOUNG OF RATS FED SUPERHEATED MILKS

Ration†	Date	Group	No. in litter	Av. wt. of litter of 4 at 4 days	Av. gain per rat per week, 4–16 days	Av. gain per rat per week 16–24 days	Mother, weight gain or loss	Remarks
				gms.	gms.	gms.	Per cent	
Evap. milk, 50 cc.	12/15	A_1	4	32	7.6			Severe convulsions on 15th day. All died.
Evap. milk, 50 cc. + 0.3 gms. autoclaved autolyzed yeast*	3/19	A_2	4	30	5.9		+11.4	Convulsions on 12th day. All dead on 15th day.
Evap. milk, 50 cc. + 0.6 gms. autoclaved autolyzed yeast.*	4/30	A_3	4	30	7.3		+13.8	Convulsions beginning on 13th day All dead on 20th day.
Evap. milk, 50 cc. + 0.6 gms. autolyzed yeast.*	4/15	A_4	4	34	15.7	21.	+13.5	
Evap. milk 50 cc. + 20 cc. wheat embyro ex.	5/12	A_5	4	36	20.	23.	+− 0	
Evap. milk 55 cc.	7/15	A_6	4	34	7.9		+16.4	Convulsions beginning on 15th day All dead on 18th day.
Evap. milk 60 cc.	7/14	A_7	4	34	11.3	17.	+9.4	Convulsions on 17th day. All recovered.
Evap. milk 65 cc.	7/14	A_8	4	32	12.	13.3	+6.2	Convulsions on 16th day. All recovered.

* Daily

TABLE I. (*Continued*)

Ration†	Date	Group	No. in litter	Av. wt. of litter of 4 at 4 days	Av. gain per rat per week, 4–16 days	Av. gain per rat per week 16–24 days	Mother, weight gain or loss	Remarks
				gms.	gms.	gms.	per cent	
Evap. milk 70 cc.	7/10	A_9	4	34	12.2	10.	+9.5	Convulsions on 17th day. All recovered.
Evap. milk 75 cc.	7/14	A_{10}	4	32	20.	12.9	+1.7	Convulsions on 19th day. One died on 24th day.
Dried milk, spray process	3/23	B_1	6	37	4.3			Convulsions on 15th day. All dead dead on 18th day.
Dried milk, spray process	6/16	B_2	4	38	8.3	4.7		Screaming and convulsions observed on 10th day to 24th day. Young miserable looking. None died.
Dried milk, spray process + 0.6 gms. autolyzed yeast*	6/14	B_3	4	39	8.3	7.2		Typical screaming convulsions observed on 15th to 21st day.
Dried milk, spray process+0.6 gms. autolyzed yeast*	10/30	B_4	4	36	9.	18.		Typical screaming convulsions on 18th and 19th days. Recovered.
Dried milk, spray process+0.9 gms. yeast*	11/2	B_5	4	32	15.1	18.6	+3.6	
Dried milk, roller process	4/7	C_1	6	42	12.4	14.3		

TABLE I. (*Continued*)

Ration†	Date	Group	No. in litter	Av. wt. of litter of 4 at 4 days	Av. gain per rat per week, 4–16 days	Av. gain per rat per week 16–24 days	Mother, weight gain or loss	Remarks
Dried milk, roller process	12/15	C_2	4	40	11.8	10.5		
Dried milk, roller process+0.6 gms. autolyzed yeast*	12/15	C_3	4	39	12.2	17.2		
Dried milk, roller process+0.9 gms. yeast*	11/1	C_6	4	61	16.1	21.1		
Desiccated albumin milk	1/30	D_1	4	36	8.8			Convulsions on 17th day. All dead on 18th day.
Desiccated albumin milk	3/19	D_2	4	42	8.4		+15.3	Convulsions on 16th day. All dead on 20th day.
Desiccated albumin milk+20 cc. wheat embryo extract	4/15	D_3	4	34	18.	21.	+4.6	One died on 14th day. Cause unknown. No convulsions.

† The standard "Hartwell" ration consisted of: dry white bread 30 gms., commercial casein 12 gms., evaporated milk 50 cc., cod liver oil 1.5 cc., iron citrate 0.05 cc. (6% sol.) and potassium iodide 0.1 cc. (2% sol.). The milk in the other rations was substituted on the basis of an equivalent amount of protein.

* Daily.

TABLE II.

THE DEVELOPMENT OF SUCKLING YOUNG OF RATS FED BOILED, PASTEURIZED AND RAW MILKS

Ration†	Date	Group	No. in litter	Av. wt. of litter of 4 at 4 days	Av. gain per rat per week, 4–16 days	Av. gain per rat per week, 16–24 days	Mother, weight gain or loss	Remarks
				gms.	gms.	gms.	Per cent	
Commercial Pasteurized Dairy 1	5/1	E_1	6	42	7.3	13.		Convulsions beginning on 15th day. All recovered.
Commercial Pasteurized Dairy 1	6/16	E_2	6	36	7.2	3.2		Convulsions on 18th day. 2 died on 23rd day.
Commercial Pasteurized Dairy 1	12/16	E_3	4	38	13	7.4		Screaming, convulsions observed in one, 20th day. Appearance of litter—fair.
Com. Past. Dairy 1—0.6 gms. autolyzed yeast*	12/15	E_4	4	40	14.1	19.2		Excellent condition.
Home Past. Dairy 1, open method B	6/17	F_1	6	48	13.5	15.8		
Home Past. Dairy 1 closed method A	6/16	F_2	4	55	16.3	21.		
Home Past. Dairy 2 closed method A	5/24	F_3	6	44	12.5	21.		

† Standard "Hartwell" ration.
* Daily

TABLE II. (*Continued*)

Ration	Date	Group	No. in litter	Av. wt. of litter of 4 at 4 days	Av. gain per rat per week, 4–6 days	Av. gain per rat per week, 16–24 days	Mother, weight gain or loss	Remarks
Quickly boiled, cooled in 3 qt. lots. Dairy 2	5/20	G_1	4	34	10.9	9.6	+13	Slight convulsions on 21st ayd. All recovered.
Quickly boiled, cooled in 3 qt. lots. Dairy 2	6/18	G_2	4	38	7.9		+−0	Convulsions on 13th day. Mother killed all on same day.
Quickly boiled, cooled in 3 qt. lots. Dairy 2	7/11	G_3	4	34	10	−2.2	+11	Convulsions on 19th day. 2 died.
Quickly boiled, cooled in 1 pt. lots. Dairy 1	7/4	G_4	6	41	11	16		Slight convulsions 16th and 17th day; one died.
Quickly boiled in 100 cc. lots. Dairy 2	6/15	G_7	4	42	14.9	14.9	+−0	
Raw milk, Dairy 2	6/14	G_8	4	30	8.7	13	+17	
Stock Rats. Av. of 3		H_1	4	36	12.9	17.1	+1.2	

At the higher levels the convulsions were less severe, and there were fewer deaths. These results with the American product are very comparable to those reported by Hartwell with English products, and lead one to conclude that a fairly large proportion of the antineuritic vitamin is destroyed in the process of preparing evaporated milks.

Evans and Burr (7), Macy, Outhouse, *et al.* (8), and Sure (9) have observed that a much larger amount of the antineuritic vitamin is needed for lactation than for growth. The latter investigator has estimated that fully 60% of the vitamin B in the food of the mother is dissipated in the metabolism and transfer to the milk. The untoward effect of too little is manifested in the young by the symptoms described, and in the mother by loss in weight, amounting to between 20% and 30%. Our results with the young of mothers receiving diets deficient in the antineuritic vitamin are comparable with those of other investigators. The mother animals, however, in our groups did not suffer the losses in weight noted by Sure. Instead these animals either maintained their weight or gained during the lactation period. Indeed, there seemed to be little if any relation between the amount of the antineuritic vitamin of the food indicated by the development of convulsions in the young, and the gain in weight of the mother. This is shown particularly with the rations which have included the different levels of evaporated milk. The early development of convulsions in the young was co-existent with good gains in the mother. In other groups normal development of the young was accompanied by stationary weights or gains in weight.

Our results with dried milks are not comparable with those of Hartwell (10). While we obtained evidence of considerable destruction of the antineuritic vitamin by the spray process and little evidence of the destruction by the roller process, she found that milks prepared by either process compared favorably with "fresh" milk. There were, however, some variations in her results which she suggests may be due to individual differences in the manufacture.

Comment

The changes incident to the destruction of the antineuritic vitamin in heat treated milk seem to be related both to the time of exposure to the high temperatures and to the degree of aeration during the process. A high temperature for a short period may be less detrimental than a lower temperature for a longer period, depending in part apparently on the time of exposure to air. One is reminded that these are the conditions

influencing the destruction of vitamin C, and that milk dried by the roller process gave less evidence of the destruction of the antiscorbutic vitamin than that dried by the spray process (11). There are undoubtedly other factors, such as the reaction and consistency of the medium which are concerned with the degree and speed of destruction of the antineuritic vitamin. The influence of these, however, would seem to be largely ruled out in this investigation, since all tests were made with milk or milk products.

Whether the lowered antineuritic vitamin content of superheated and long heat-treated milk plays any part in the nutritional disturbances of the artificially fed infant, we are not prepared to say. There is accumulating evidence to the effect that the undernourished breast fed baby may be getting too little of this vitamin for optimum physiological well being. Moore (12) has reported brain hemorrhages in the newborn, which he has attributed to an antineuritic vitamin deficiency in the diet of the mother. Sure (13), Evans and Burr (14), and Hoobler (15), quoting Macy and Outhouse, have called attention to the fact that the antineuritic vitamin of human milk may be below the margin of safety, even in apparently well nourished mothers. Hoobler has suggested that this also may be the case in many of the cow's milk mixtures used in infant feeding. An artificial feeding consisting of cow's milk, syrup, orange juice, and cod liver oil may contain "only the minimal and in some instances sub-minimal amounts of Vitamin B." The author cites hospital patients in whom the symptoms were somewhat similar to those of infants suffering from beriberi. The addition to the feeding mixtures of small amounts of substances known to be rich in the antineuritic vitamin resulted in gains in weight that were out of all proportion to the increase in food intake. Similar observations were made by Eddy (16) with marasmic infants and by Daniels, Byfield, and Laughlin (17) with a number of seemingly normal children. Whether the stimulation of growth in these cases was due to the added antineuritic vitamin or other substances contained in the mixture is not clear. The subject warrants further investigation.

Summary

The investigation has included a study by means of the Hartwell method, of the antineuritic vitamin content of various forms of heat-treated milks used in infant feeding. The milks tested have included evaporated milk, two types of dried milk, a desiccated albumin-milk, boiled milk, and milk pasteurized both by the open and closed method

(hold process.) With the exception of the milk dried by the roller process, all the superheated milks gave evidence of considerable destruction of the vitamin. The results with the open method, pasteurized milk tested, also suggested that there is some destruction of the vitamin; much less, however, than in either evaporated milk or milk dried by the spray process. Milk pasteurized by the closed method gave no evidence of the vitamin destruction. Milk heated to the boiling temperature quickly and cooled slowly seems to be somewhat affected. The results of tests with these were fairly comparable to those of the open method pasteurized milk. Milk boiled quickly and cooled quickly is little, if at all, affected. The results of the investigation suggest that temperature and aeration are important factors in determining the degree of destruction of the anti-neuritic vitamin in milk.

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